UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

INSTITUTO DE BIOCIÊNCIAS

PROGRAMA DE PÓS-GRADUAÇÃO EM GENÉTICA E BIOLOGIA MOLECULAR

MUCOPOLISSACARIDOSES: CARACTERIZAÇÃO DAS ALTERAÇÕES CARDIOVASCULARES E AVALIAÇÃO DA SEGURANÇA DO TRATAMENTO COM LOSARTANA

FABIANO DE OLIVEIRA POSWAR

Orientador: Prof. Roberto Giugliani

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Tese submetida ao Programa de Pós-Graduação em Genética e Biologia Molecular da UFRGS como requisito parcial para a obtenção do grau de Doutor em Genética e Biologia Molecular

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Sumário

Resumo

As mucopolissacaridoses (MPS) são um grupo de doenças lisossômicas causadas pelo acúmulo de glicosaminoglicanos, associadas a um acometimento multissistêmico e com significativa sobreposição clínica entre seus tipos. Os problemas cardiovasculares estão entre as manifestações mais comumente observadas e também estão entre as principais causas de morbimortalidade nesses pacientes. Recentemente, a dilatação de raiz de aorta (DRA) tem sido apontada como uma alteração frequente em diferentes tipos de MPS. Para algumas das manifestações cardiovasculares, como a DRA e as doenças valvares, não há tratamento eficaz atualmente disponível. Nesse sentido, nós inicialmente revisamos os achados ecocardiográficos de 69 pacientes com diferentes tipos de MPS (25 com MPS I, 21 com MPS II, 16 com MPS IVA e 7 com MPS VI) e confirmamos que a DRA é um achado frequente nas MPS, sendo especialmente prevalente em pacientes com MPS IVA e MPS VI. Além disso, verificamos que a terapia de reposição enzimática (TRE) parece ter pouco impacto sobre seu surgimento e progressão. Na sequência, realizamos uma avaliação mais extensa em 76 pacientes com MPS, avaliando parâmetros relacionados à hipertrofia ventricular esquerda, hipertensão pulmonar, função sistólica e condução atrioventricular. Observamos que o uso de TRE esteve associado à redução de parâmetros relacionados à presença de hipertrofia ventricular esquerda, porém com efeito limitado sobre outros parâmetros. Considerando o potencial terapêutico da losartana sobre a doença cardíaca nas MPS, verificado em modelos animais, foi desenhado um ensaio clínico de fase II, randomizado, duplo cego, com o objetivo de avaliar a segurança e a eficácia da losartana para o tratamento de manifestações cardiovasculares em pacientes com MPS IVA e VI. Os resultados obtidos na etapa inicial do estudo demonstraram um perfil de segurança favorável em pacientes com MPS IVA e trouxeram dados importantes para projetar a continuidade da avaliação da losartana como agente terapêutico nas MPS.

Palavras-chave: bloqueador de receptor de angiotensina, doenças lisossômicas, dilatação de raiz de aorta, terapia de reposição enzimática, glicosaminoglicanos.

Abstract

The mucopolysaccharidoses (MPS) are a group of lysosomal diseases caused by the accumulation of glycosaminoglycans, associated with multisystemic involvement and with significant clinical overlap among their types. Cardiovascular problems are among the most commonly observed manifestations and are also among the main causes of mortality in these patients. Recently, aortic root dilation (ARD) has been identified as a frequent abnormality in different types of MPS. For some of the cardiovascular manifestations, such as ARD and valvular diseases, there is currently no effective treatment available. In this sense, we initially reviewed the echocardiographic findings of 69 patients with different types of MPS and confirmed that ARD is a frequent finding in mucopolysaccharidoses, being especially prevalent in patients with MPS IVA and MPS VI. In addition, we found that enzyme replacement therapy (ERT) appears to have little impact on its occurrence and progression. Thereafter, we carried out a more extensive evaluation in 76 patients with MPS, evaluating parameters related to ventricular hypertrophy, pulmonary hypertension, systolic function and cardiac conduction and we observed that the use of ERT was associated with a reduction in parameters related to the presence of left ventricular hypertrophy, but with limited effect on other parameters. Considering the therapeutic potential of losartan on heart disease in MPS verified in animal models, a randomized, double-blind, phase II clinical trial was eventually designed to assess the safety and efficacy of losartan for treatment of cardiovascular disease in patients with MPS IVA and VI. The initial results showed a favorable safety profile in patients with MPS IVA and bring important data for the continuity of the evaluation of losartan as a therapeutic agent in the MPS.

Keywords: angiotensin receptor blocker, lysosomal diseases, aortic root dilation, enzyme replacement therapy, glycosaminoglycans

Capítulo 1
Introdução

1. Doenças lisossômicas

As doenças lisossômicas (DLs) são um grupo de erros inatos do metabolismo resultantes da deficiência de hidrolases, proteínas transportadoras ou outras proteínas envolvidas na função dos lisossomos (Sabatini and Adesnik 2014). Os lisossomos são organelas que desempenham um papel central em diversos processos celulares, incluindo funções degradativas como a fagocitose, autofagia e outras funções com participação na ativação de vias de sinalização, tráfego de vesículas e membranas plasmáticas, imunidade adaptativa, entre outras funções (de Duve 1964; Settembre et al. 2013).

Atualmente são descritas 66 DLs, relacionadas a alterações de 57 genes distintos (WORLDSymposium 2018). Entre as DLs mais comuns estão relacionadas à função de hidrolases ativadas no pH ácido do lisossomo, as quais são responsáveis pela hidrólise de moléculas complexas como os esfingolipídios (esfingolipidoses) e os glicosaminoglicanos (mucopolissacaridoses, MPS), resultando em acúmulo dessas substâncias e consequente disfunção lisossomal, sendo também conhecidas como "doenças lisossômicas de depósito" (DLDs). A figura 1 sintetiza o mecanismo fisiopatológico das DLs.

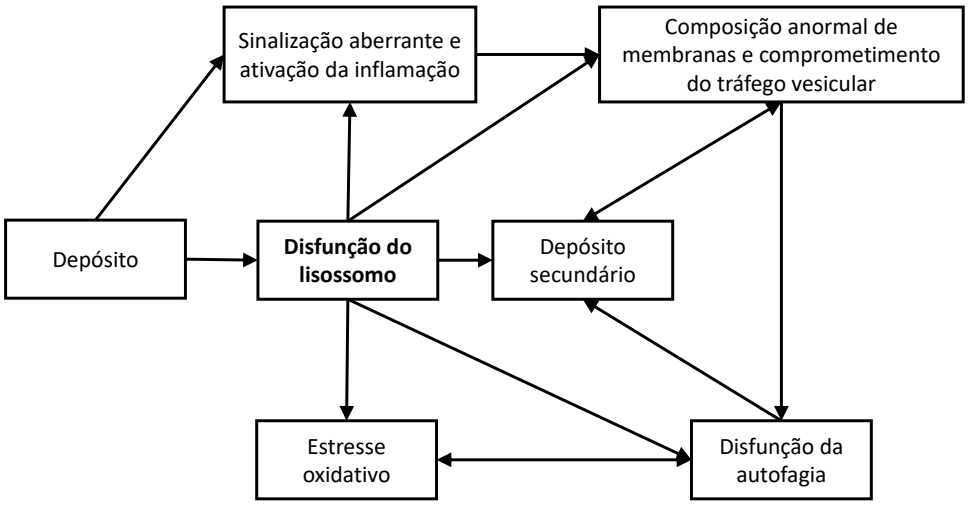

Figura 1. Fisiopatologia das DLs. Adaptado de Fecarotta et al. 2018.

As primeiras doenças de depósito foram descritas como entidades clínicas no final do século XIX (Gaucher 1882). Pouco após a descoberta do papel dos lisossomos em sua fisiopatologia (Hers 1963), a possibilidade de se encontrar tratamentos eficazes para esse grupo de doenças tem mobilizado os pesquisadores (de Duve 1964). Atualmente, cerca de um terço

das DLs tem um tratamento específico, incluindo a maior parte das DLs mais comuns, tornando-se condições em que a identificação e tratamento precoces têm alto impacto no prognóstico, o que vem motivando a inclusão de algumas delas em programas públicos de triagem neonatal (Platt et al. 2018; Arunkumar et al. 2020) e a incorporação de procedimentos terapêuticos e produtos comerciais nos sistemas públicos de saúde, ainda que sob exigência de acompanhamento em centros de referência (Caetano et al. 2019). O anexo 1 desta tese inclui uma revisão sobre os diversos aspectos sobre o diagnóstico e o tratamento dessas condições e o anexo 2 resume novas terapias direcionadas ao sistema nervoso central, uma área ainda com poucas opções terapêuticas nas doenças lisossomais de um modo geral. Nos itens a seguir será dado atenção especial a um grupo específico de doenças lisossomais, as MPS.

2. Mucopolissacaridoses (MPS)

As MPS são um grupo de DLs causadas pelo acúmulo de glicosaminoglicanos (GAGs, anteriormente conhecidos como mucopolissacarídeos). Entre os tipos atualmente descritos de MPS, onze são decorrentes da deficiência de enzimas lisossomais, enquanto uma outra condição mais recentemente descrita decorre da alteração de uma proteína envolvida na fusão endossomo-lisossomo e autofagossomo-lisossomo (Kondo et al. 2016). Entre as MPS, apenas a MPS II tem herança ligada ao X; todas as demais são de herança autossômica recessiva.

Os glicosaminoglicanos são heteropolissacarídios, que, juntamente com proteínas fibrosas, compõem a matriz extracelular. Estruturalmente, os GAGs são caracterizados por terem um dissacarídio de repetição central, sendo um dos monossacarídios a N-acetilglicosamina ou N-acetilgalactosamina e o outro o ácido D-glicurônico ou o ácido L-idurônico. Frequentemente, uma ou mais hidroxilas do aminoaçúcar recebe um resíduo de sulfato (figura 2). Entre os principais GAGs estão o hialuronato (ou hialuronan), a condroitina-6-sulfato (C6S), a condroitina-4-sulfato (C4S), o queratan sulfato (QS), o heparan sulfato (HS) e o dermatan sulfato (DS). O hialuronato ocorre em até 50.000 repetições produzindo uma solução altamente viscosa presente no líquido sinovial das articulações, no humor vítreo, e na matriz extracelular da cartilagem e dos tendões. Os demais glicosaminoglicanos ocorrem em polímeros muito mais curtos e associados a certas proteínas de ligação associadas à membrana celular ou à matriz celular como parte de macromoléculas chamadas proteoglicanos, as quais contribuem para a força tênsil de cartilagens, ligamentos, tendões e parede da aorta, estando também presentes na pele, vasos sanguíneos, válvulas cardíacas, córneas e ossos (Nelson et al. 2014).

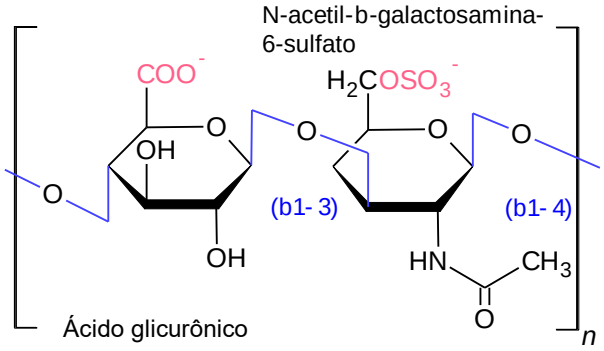

Figura 2 – Estrutura do polímero de condroitina-6-sulfato, um glicosaminoglicano acumulado na mucopolissacaridose IVA. Adaptado de Nelson et al. 2014.

O ciclo de síntese e degradação dos GAGs é importante para a manutenção das diversas estruturas em que essas macromoléculas são expressas. A degradação dos GAGs envolve a participação de diferentes enzimas, incluindo exoglicosidases (alfa-L-iduronidase, beta-glicuronidase, alfa-N-acetilglicosaminidase, beta-galactosidase, beta-hexosaminidases A, B e S), exossulfatases (iduronato-2-sulfatase, N-acetilgalactosamina 4-sulfatase, heparan-N-sulfatase, N-acetilglicosamina-6 sulfatase, N-acetilgalactosamina-6-sulfatase, glicuronato 2-sulfatase), uma acetiltransferase (acetil-CoA:alfa-glicosaminida n-acetiltransferase) e uma endoglicosidase (hialuronidase) (Neufeld and Meunzer 2001). Como uma mesma função pode ser exercida por mais de uma enzima, a deficiência isolada de uma delas nem sempre causa acúmulo de GAGs. Atualmente, já foram descritas deficiências de onze dessas enzimas em seres humanos que resultam em acúmulo de GAGs e a expressão clínica como uma mucopolissacaridose (tabela 1).

Tabela 1 – MPS atualmente descritas, incluindo a MPS *plus*.

Tipo de MPS	**Gene**	**Produto gênico deficiente**	**GAGS acumulados**
MPS I	*IDUA*	α-L-iduronidase	DS, HS
MPS II	*IDS*	Iduronato sulfatase	DS, HS
MPS IIIA	SGSH	Heparan-n-sulfatase	HS

MPS IIIB	NAGLU	α-N-acetilglucosaminidase	HS
MPS IIIC	*HGSNAT*	Acetil-CoA-N:α-glicosaminida acetiltransferase	HS
MPS IIID	*GNS*	N-acetilglicosamina-6- sulfatase	HS
MPS IVA	*GALNS*	N-acetilgalactosamina-6-sulfato sulfatase	QS, C6S
MPS IVB	*GLB1*	Beta-galactosidase	QS
MPS VI	*ARSB*	Arilsulfatase B	DS, C4S
MPS VII	*GUSB*	Beta-glicuronidase	DS, HS, C4S, C6S
MPS IX	*HYAL1*	Hialuronidase	Hialuronan
MPSPS	*VPS33A*	Proteína de triagem vacuolar 33	DS, HS

Como nas outras DLs, o mecanismo fisiopatológico proposto envolve o acúmulo de GAGs nos lisossomos e o desencadeamento de uma cascata de eventos que incluem a disfunção dos lisossomos e o prejuízo a diversas funções celulares (Fecarotta et al. 2020) (figura 3). No caso particular das MPS, há tipicamente o envolvimento de diferentes tecidos em que os glicosaminoglicanos são abundantes, como os ossos, articulações, válvulas cardíacas, cérebro, pele, vísceras e tecidos moles, resultando em uma ampla variedade de sinais e sintomas.

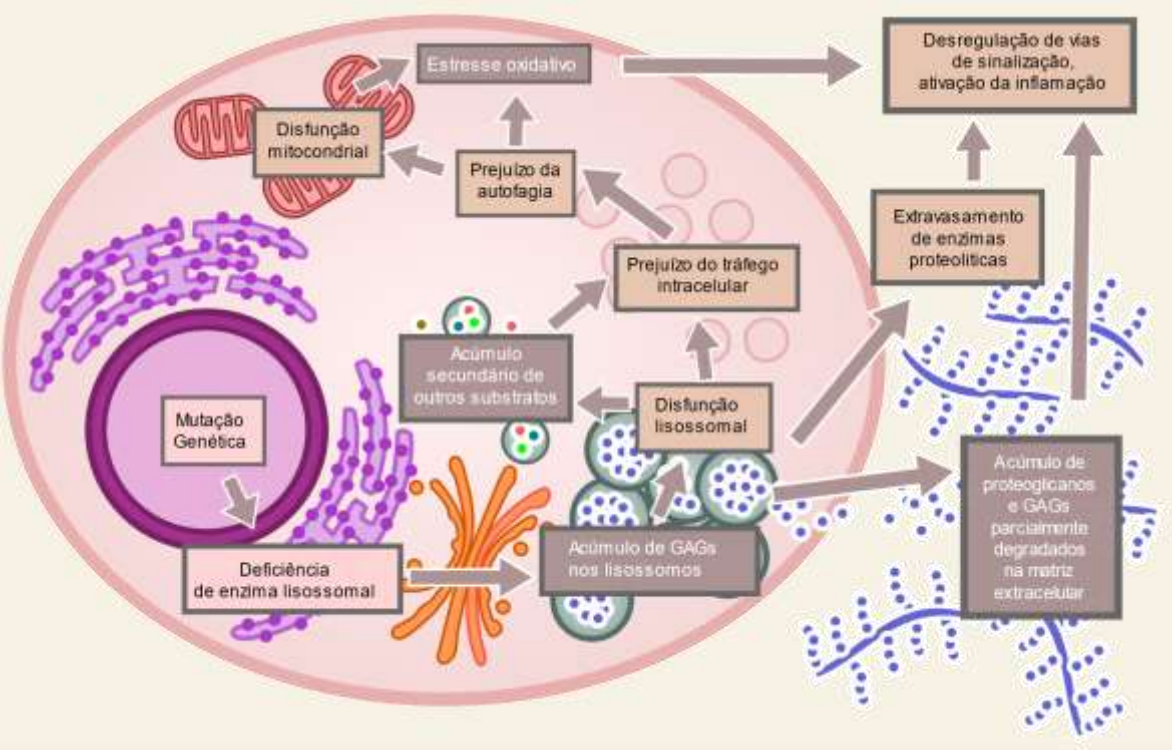

Figura 3**.** Mecanismo fisiopatológico das MPS. Adaptado de Fecarotta et al. 2020.

2.1. Manifestações clínicas

As MPS são doenças multissistêmicas e com significativa sobreposição clínica entre seus tipos, sendo comumente associadas à organomegalia, face grosseira, opacidade de córnea, baixa estatura e deficiência intelectual. Por outro lado, há também características clínicas distintas entre as MPS, o que reflete os papeis específicos das enzimas relacionadas a degradação dos GAGs. A tabela 2 resume algumas das principais manifestações clínicas das MPS.

Tabela 2 – Manifestações clínicas das MPS

Manifestação clínica	**Tipo de MPS**											
	I	II	VI	VII	IIIA	IIIB	IIIC	IIID	IVA	IVB	IX	PS
Face grosseira	+++	+++	+++	++	+	+	+	+	+	+	-	+++
Organomelia	+++	+++	+++	+	+	+	+	+	+	+	-	++
Manifestações cardiovasculares	+++	+++	+++	+++	+	+	+	+	++	+	-	++
Alterações esqueléticas	++	++	++	++	+	+	+	+	+++	+++	+	++
Rigidez articular	+++	+++	+++	+++	+	+	+	+	-	-	-	++
Deficiência intelectual	++	++	+	-	+++	+++	+++	+++	-	+	-	+++
Baixa estatura	++	++	++	++	-	-	-	-	+++	++	+	+++
Hérnia umbilical e/ou inguinal	+++	+++	+++	++	-	-	-	-	+	+	-	-
Opacidade de córnea	+++	-	+++	++	-	-	-	-	+	+	-	-
Macroglossia	+++	+++	+++	++	-	-	-	-	-	-	-	++
Proteinúria	-	-	-	-	-	-	-	-	-	-	-	++
Citopenia	-	-	-	-	-	-	-	-	-	-	-	++

Os sinais (-, +, ++ e +++) indicam se uma determinada alteração está mais frequentemente ausente em um tipo específico de mucopolissacaridose (-) ou se está frequentemente presente em intensidade leve (+), moderada (++) ou grave (+++). PS: MPS plus. Adaptado de Poswar et al. 2017 e Kubaski et al. 2020.

Como podemos observar, as MPS apresentam importantes particularidades em relação a suas manifestações clínicas, cujo reconhecimento é essencial para o estabelecimento do diagnóstico. O anexo 3 desta tese discute os métodos diagnósticos disponíveis, incluindo a triagem de pacientes de alto risco com base em seus sinais e sintomas clínicos. Nas próximas seções, detalhamos as particularidades das MPS tipos I, II, IVA e VI e as manifestações cardiovasculares das MPS no geral.

2.1.1 Manifestações clínicas da mucopolissacaridose I

A mucopolissacaridose tipo I decorre da deficiência da enzima alfa-L-iduronidase (IDUA), com consequente acúmulo de HS e DS (Neufeld and Muenzer 2014). A MPS I é classicamente

dividida em três apresentações clínicas, de acordo com a gravidade. A forma mais grave, a síndrome de Hurler, tem sua apresentação no primeiro ano de vida, sendo associada a sintomas respiratórios, hérnia inguinal e umbilical, hipertricose e face grosseira (figura 4). A síndrome de Hurler tipicamente se associa a um declínio cognitivo progressivo, que se torna evidente até os 18 meses de vida. Um grande espectro de alterações esqueléticas (conhecido como disostose múltipla) também se torna evidentes e resultam em baixa estatura. Cardiopatia, hepatoesplenomegalia e opacificação da córnea são outras manifestações presentes na maioria dos casos (Beck et al. 2014). Uma forma atenuada, a síndrome de Scheie (anteriormente chamada MPS tipo V), caracteriza-se por uma apresentação mais tardia, com rigidez articular, baixa estatura, doença cardíaca valvar e opacidade corneana, mas sem neurodegeneração (Vijay and Wraith 2005). A síndrome de Hurler-Scheie é o termo empregado para formas intermediárias, em que há um comprometimento somático grave, semelhante ao da síndrome de Hurler, mas com a função cognitiva preservada ou apenas levemente comprometida. O anexo 4 desta tese apresenta uma revisão sobre a MPS I.

Figura 4**.** Ilustração de uma criança com face grosseira, tipicamente associada aos casos graves de MPS I, II e VI. As características incluem: macrocefalia, bossa frontal, ponte nasal larga, achatamento da face média, lábios grossos, dentes pequenos e afastados e macroglossia. A opacidade da córnea representada na ilustração é verificada nas MPS I e VI, mas não na MPS II.

2.1.2 Manifestações clínicas da mucopolissacaridose II

A mucopolissacaridose tipo II decorre da deficiência da enzima iduronato sulfatase (IDS), resultando também em acúmulo de HS e DS, como na MPS I (Neufeld and Muenzer 2014). É

uma condição de herança ligada ao X, afetando quase exclusivamente indivíduos do sexo masculino, com raros casos em mulheres. O espectro de gravidade é semelhante ao descrito para a MPS I, com formas mais graves de início precoce e associados a neurodeneração (neuronopáticas) e formas mais atenuadas, de aparecimento mais tardio, com preservação da função cognitiva (não neuronopáticas) (Young et al. 1982). As manifestações clínicas comuns às formas neuronopáticas e não neuronopáticas incluem baixa estatura, macrocefalia com ou sem hidrocefalia comunicante, macroglossia, voz rouca, perda auditiva, hepatoesplenomegalia, disostose múltipla, doença cardíaca e síndrome do túnel do carpo (Schwartz et al. 2007). Apesar de acumularem os mesmos tipos de GAGs, há também algumas diferenças clínicas relevantes entre a MPS I e a MPS II. Pacientes com MPS II tipicamente não apresentam opacidade da córnea e crianças com a forma grave de MPS II tendem a apresentar um padrão de comportamento agressivo, que é menos frequentemente observado na MPS I (Cross and Hare 2013). Acredita-se que algumas dessas diferenças possam ser atribuídas às diferenças entre as porções expostas dos GAGs parcialmente degradados nessas duas condições, com potencial de ativar diferentes processos celulares (Gaffke et al. 2020).

2.1.3 Manifestações clínicas da mucopolissacaridose IVA

A mucopolissacaridose tipo IVA é um tipo de MPS que decorre da deficiência da enzima N-acetilgalactosamina-sulfato sulfatase (GALNS), com consequente acúmulo de QS e C6S. O QS é responsável por cerca de 25% da composição dos GAGs nas cartilagens de adultos e a redução da sua degradação resulta em seu acúmulo no interior dos condrócitos, resultando em um quadro de displasia esquelética, com acometimento de epífises, metáfises e corpos vertebrais (Tomatsu et al. 2014). Na MPS IVA (mas não na MPS IVB), o acúmulo de C6S não degradado também contribui para o agravamento do dano às cartilagens e outras estruturas corporais (Shimada et al. 2014). Há grande variação clínica entre pacientes com MPS IVA, resultando em pacientes com quadros mais atenuados ou mais graves (Neufeld and Muenzer 2014).

Além dos sintomas esqueléticos, pacientes com MPS IVA apresentam alterações das vias aéreas superiores e inferiores que incluem estreitamento da traqueia, apneia do sono, otite média recorrente, pneumonia recorrente, hipertrofia de adenoides, tonsilas, e cordas vocais. (Harmatz et al. 2013). Outras manifestações clínicas em demais sistemas corporais incluem

instabilidade cervical, compressão da medula cervical ou toracolombar, perda auditiva, alterações dentárias e um conjunto de sinais e sintomas cardiovasculares (Montaño et al. 2007).

2.1.4 Manifestações clínicas da mucopolissacaridose VI

A mucopolissacaridose tipo VI (MPS VI) decorre da deficiência da enzima arilsulfatase B (ARSB), com consequente acúmulo de DS e C4S (Neufeld and Muenzer 2014; Harmatz 2017). Isso resulta em um quadro clínico também semelhante ao descrito para a MPS I, porém sem envolvimento primário do sistema nervoso central, o que é explicado pela ocorrência de acúmulo de DS em ambas as condições, sem, no entanto, ocorrer acúmulo de HS na MPS VI. O papel do C4S nas manifestações clínicas é menos evidente. A rigidez articular é a principal manifestação clínica, estando presente em casos graves ou atenuados. Além disso, pacientes tipicamente apresentam face grosseira, dolicocefalia, opacidade corneana, baixa acuidade visual, macroglossia, atraso de erupção dentária. Alterações esqueléticas incluem geno valgo, pectus carinatum, cifoescoliose, lordose lombar, displasia de quadril, baixa estatura, mãos em garra, entre outros. Infecções de vias aéreas superiores recorrentes são comuns, assim como otites médias, que podem resultar em deficiência auditiva. Alterações cardiovasculares são frequentes e mais graves, quando comparadas a outras MPS (Azevedo et al. 2004; Golda et al. 2012).

2.1.5 Manifestações cardiovasculares das mucopolissacaridoses

Os problemas cardíacos estão entre as manifestações mais comumente observadas em pacientes com MPS, sendo descritos em todos os tipos de MPS, com exceção da MPS IX. Além disso, juntamente com as infecções respiratórias e a doença pulmonar restritiva, estão entre as principais causas de mortalidade nas MPS (Tan et al. 1992; Jones et al. 2009). A idade de início dos sinais e sintomas cardiovasculares é variável, sendo menor em pacientes com síndrome de Hurler e formas neuronopáticas de MPS II (Fesslová et al. 2009) e maior em pacientes com MPS III (Wilhelm et al. 2018).

Entre as manifestações cardiovasculares mais comuns em pacientes com MPS, estão as doenças valvares, as quais são especialmente prevalentes nas MPS em que há acúmulo de DS, isto é, nos tipos I, II,VI e VII (Tan et al. 1992; Leal et al. 2010; John et al. 2011; Braunlin et al. 2011). Tanto em humanos como em modelos animais de MPS I, é observado nas valvas uma expansão das camadas esponjosa e fibrosa por células claras contendo GAGs associado à

escassez e desarranjo de fibras de elastina (Braunlin et al. 2006). O envolvimento valvular está frequentemente associado à regurgitação, em vez de estenose, e as válvulas mais acometidas são a mitral e a aórtica (Chen et al. 2005). A regurgitação valvar, por sua vez, pode resultar em sobrecarga e/ou hipertrofia do ventrículo esquerdo, e em estágios tardios, pode ocorrer disfunção sistólica. Déficit de relaxamento também pode ocorrer (disfunção diastólica) (Fesslová et al. 2009; Leal et al. 2010). Em pacientes adultos, procedimentos cirúrgicos são frequentemente necessários para tratamento da doença valvar (Arn et al. 2012).

A hipertrofia do ventrículo esquerdo é outro achado frequentemente observado em diferentes tipos de MPS (Lin et al. 2018). Ao exame por microscopia eletrônica, a hipertrofia ventricular é associada à presença de células espumosas no espaço intersticial e inclusões em corpos de zebra nos cardiomiócitos (Izumi et al. 2018).

Doença arterial coronariana, com estreitamento ou oclusão das artérias subepicárdicas também ocorre, em especial na MPS I e MPS II (Braunlin et al. 2011). Na maioria dos casos descritos, a doença coronariana é assintomática (Soliman et al. 2007; Kampmann et al. 2011), com raros relatos de pacientes com angina (Craig 1954). Apesar disso, a doença arterial coronariana tem sido relacionada à ocorrência de morte súbita nesses pacientes conforme avalições de necrópsias (Lin et al. 2005; Sohn et al. 2012). Na histopatologia, observa-se proliferação da mioíntima por células claras e alteração nas fibras de colágeno (Braunlin et al. 2006).

A hipertensão arterial é observada frequentemente na MPS I e MPS II, sendo rara em outros tipos de MPS (Taylor et al. 1991). Apesar disso, deve-se considerar que mesmo indivíduos com outros tipos de MPS, como a MPS VI, possuem maior prevalência de alguns dos fatores envolvidos na etiologia da hipertensão arterial, como a apneia obstrutiva do sono (Braunlin et al. 2006; Golda et al. 2012). Em pacientes com MPS II, seguidos no estudo *Hunter Outcome Survey*, 25% apresentavam níveis de pressão arterial acima da referência para a idade, embora apenas 2% dos pacientes tivessem informado uma história de hipertensão arterial sistêmica (Kampmann et al. 2011). A hipertensão pulmonar também é descrita e pode ocorrer em até 50% dos pacientes com MPS tipo VI (John et al. 2011).

Outras manifestações cardiovasculares menos frequentes incluem a fibroelastose do endocárdio, uma apresentação rara e grave da doença cardíaca restritiva, descrita em algumas crianças com MPS (Fong et al. 1987), e o aneurisma do ventrículo esquerdo, reportado raramente em adultos com MPS (Oudit et al. 2007; Cabrera et al. 2012).

2.1.6 Dilatação da raiz da aorta

Os grandes vasos podem ter espessamento de suas paredes e estarem estreitados ou dilatados. Foi registrado em adultos com forma atenuada de MPS I uma maior ocorrência de rigidez da aorta (Nemes et al. 2008). Por outro lado, em modelos animais de MPS I, é frequentemente encontrada uma significativa dilatação da raiz da aorta (DRA), resultando no apagamento da crista sinotubular (Braunlin et al. 2006). Embora as primeiras análises não tenham conseguido encontrar o mesmo padrão de DRA em humanos (Braunlin et al. 2006), outros autores mais recentemente descreveram que esse padrão também ocorre em pacientes com diferentes tipos de MPS; especialmente MPS IVA (Kampmann et al. 2016a; Bolourchi et al. 2016a; Bolourchi et al. 2016b).

2.2. Tratamento das mucopolissacaridoses

Duas estratégias principais estão disponíveis atualmente para o tratamento das MPS visando a modificação do curso da doença: a terapia de reposição enzimática (TRE) por via intravenosa e o transplante de células tronco hematopoiéticas (TCTH). Ambas as estratégias consistem em restaurar a atividade enzimática seja por meio de suplementação de uma enzima recombinante produzida em laboratório (TRE) ou pela infusão de células tronco hematopoiéticas de um doador saudável (TCTH).

A TRE é a principal modalidade de tratamento para a maior parte das MPS. Atualmente, diferentes produtos de TRE estão disponíveis para as MPS tipo I, II, IVA, VI e VII e há ensaios clínicos em andamento para outros tipos de MPS. Contudo, a TRE, apesar de poder reduzir os GAGs a níveis próximos da normalidade, apresenta importantes limitações quanto à sua capacidade de reverter ou mesmo impedir a progressão de muitas das manifestações clínicas das MPS, incluindo diferentes manifestações neurológicas, esqueléticas, cardiovasculares e oculares (Parini and Deodato 2020).

Há diferentes motivos para explicar a eficácia limitada da TRE. As enzimas recombinantes atualmente disponíveis são incapazes de atravessar a barreira hematoencefálica e atingir as células alvo no sistema nervoso central, o que limita seu papel nas formas neuronopáticas das MPS. Além disso, certos tecidos e órgãos afetados nas MPS, como a córnea e os ossos, são pouco irrigados, limitando a distribuição da enzima infundida por via endovenosa. Por fim, entende-se atualmente que a patogênese da mucopolissacaridose envolve não só o acúmulo de GAGs, mas também mudanças significativas em processos celulares, as quais poderiam já estar

estabelecidas de uma maneira tal que a redução do acúmulo de GAGs por si seria incapaz de revertê-las (Gaffke et al. 2020).

O TCTH, por sua vez, apresenta uma vantagem significativa sobre a TRE, por sua capacidade de preservar a função cognitiva quando realizado precocemente, ao menos em pacientes com a forma grave de MPS I, tendo sido o primeiro tratamento utilizado com sucesso para essa condição ainda em 1981 (Hobbs 1981). Tal efeito sobre o sistema nervoso central se deve à reposição das células da micróglia a partir dos macrófagos derivados das células do doador. Ainda assim, a progressão de certas manifestações esqueléticas, cardiovasculares e oculares também tem sido observada em pacientes tratados com TCTH (Taylor et al. 2019). Além disso, apesar de seu uso poder ser considerado nas formas atenuadas de MPS I e em outros tipos de MPS, a morbimortalidade associada ao transplante limita sua utilização nessas situações.

O anexo 5 desta tese inclui uma revisão de estudos clínicos relacionados ao desenvolvimento de novos tratamentos para as MPS, enquanto o anexo 6 inclui uma revisão aprofundada especificamente no uso da TRE intratecal e intracerebrovascular e o anexo 7 apresenta uma prova de conceito da terapia gênica não viral como estratégia para expressão enzimática em longo prazo. Discutiremos na seção a seguir o papel de um produto específico, a losartana, o racional de seu uso como agente terapêutico nas MPS e os resultados obtidos em estudos não clínicos.

3. Losartana

3.1. Descrição química

A losartana é um antagonista não peptídico dos receptores AT1, integrante da classe dos bloqueadores de receptores de angiotensina (BRAs). Os BRAs atuam de forma semelhante aos inibidores da enzima conversora de angiotensina (IECAs) no controle da pressão arterial (Goa and Wagstaff 1996). Por outro lado, considerando que a enzima conversora da angiotensina não é a única responsável pela geração de angiotensina II, *in vivo*, não se pode assumir que os BRAs tenham propriedades terapêuticas idênticas aos IECAs (figura 5). A losartana é convertida no fígado em seu metabólito ativo, E3174, com a substituição do radical hidroximetila no anel imidazólico pelo radical carboxílico, responsável pela maior parte do efeito de bloqueio dos receptores AT1 (figura 6),

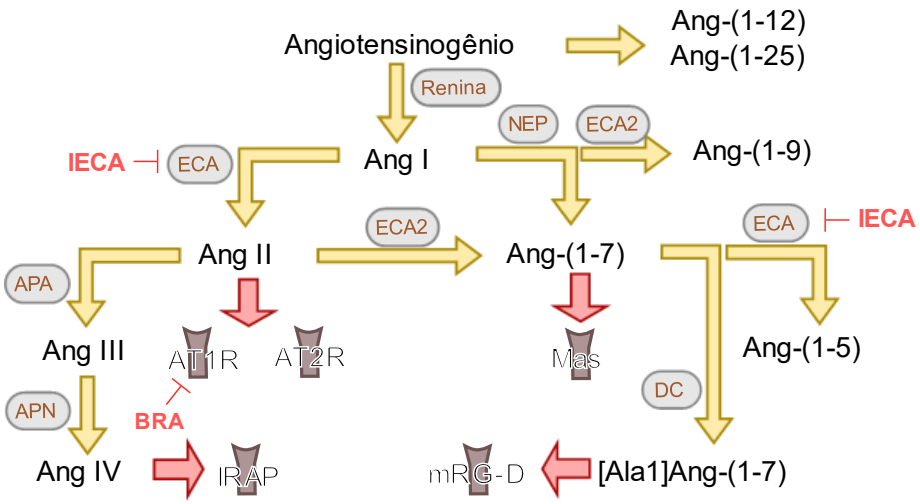

Figura 5. Sistema renina-angiotensina e a cascata de processamento dos peptídeos de angiotensina, indicando local de ação dos inibidores da enzima conversora de agiotensina (IECA) e dos bloqueadores do receptor de angiotensina II (BRA). As setas amarelas indicam a ação de diferentes reações enzimáticas e as setas vermelhas, a ligação dos peptídeos aos receptores. Ang: angiotensina. AT1R: receptor de angiotensina II tipo I. AT2R: de angiotensina II tipo II. APA: aminopeptidase A. APN: aminopeptidase N. DC: descarboxilase do ácido aspártico. ECA: enzima conversora da angiotensina. ECA2: enzima conversora da angiotensina 2. IRAP: aminopeptidase regulada por insulina. NEP: neprilisina. mRG-D: receptor acoplado a proteína G relacionado ao Mas. Adaptado de (Chappell 2016)

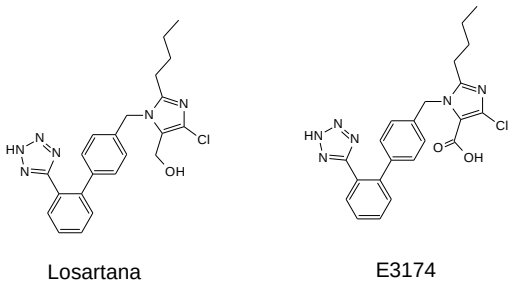

Figura 6. Estrutura química da losartana e do metabólito ativo E3174. Há substituição de um radical hidroximetila no anel imidazólico pelo radical carboxílico.

3.2. A losartana como potencial tratamento para as manifestações cardiovasculares das mucopolissacaridoses

A angiotensina II é um potente vasoconstritor que sinaliza através do receptor de angiotensina II tipo I (AT1) e o receptor de angiotensina II tipo II (AT2), sendo AT1 amplamente conhecido por regular a expressão do gene TGF- β1 em modelos animais de hipertrofia cardíaca (Everett et al. 1994). O receptor AT1 pode incrementar a produção dos ligantes e receptores de TGF-β, assim como ativadores, como a trombospondina-1 (Zhou et al. 2006), que é um potente ativador da via de sinalização do TGF-β. Em contrapartida, o receptor

AT2 induz efeitos opostos ao receptor AT1, atenuando a proliferação e inibindo a inflamação nas lesões vasculares (Wu et al. 2001). O receptor AT1 e, em menor intensidade o receptor AT2, também desempenham um papel na regulação da síntese de proteoglicanos (Shimizu-Hirota et al. 2001).

A superfamília de proteínas TGF-β tem sido envolvida em múltiplos processos celulares, incluindo a regulação da proliferação celular, a diferenciação, a reparação e remodelamento de tecidos, e a apoptose. A desregulação de TGF-β tem sido associada em algumas doenças humanas como doenças autoimunes, síndrome de Marfan, além de outros processos anormais como a dilatação cardíaca, aneurisma aórtico e a invasividade de tumores (Kalluri and Han 2008; Krstic and Santibanez 2014).

Nas MPS, o aumento na sinalização de TFG-β foi descrito em condrócitos de ratos e gatos com MPS VI (Simonaro et al. 2005), na aorta de camundongos MPS I (Ma et al. 2008) e em lesões vasculares de cães com MPS I (Lyons et al. 2011). Também tem sido reportado um aumento da expressão do SMAD2 fosforilado (relacionado à via de sinalização do TGFβ) em lesões coronárias e cardíacas de pacientes com MPS I (Yano et al. 2009; Yano et al. 2013).

Em um modelo animal de outra doença lisossomal (a mucolipidose), também se observou o papel da via TGFβ na patogênese das alterações da cartilagem. A ativação dessa via mediaria um aumento de C4S, com aumento da atividade da catepsina K, a qual novamente resultaria na ativação da via TGFβ, compondo um ciclo de retroalimentação positiva. Esse ciclo seria potencialmente interrompido pelo bloqueio farmacológico de TGFβ (Flanagan-Steet et al. 2018).

Neste sentido os BRAs, como a losartana, inibem seletivamente o receptor AT1 (Burnier 2001) atenuando a sinalização de TGFβ, reduzindo a expressão de ligantes, receptores e ativadores de TGFβ (Fukuda et al. 2000; Naito et al. 2004) (Figura 7). Consequentemente, a sinalização em *downstream* de TGF-β poderia atenuar a atividade de outras proteínas, entre estas as metaloproteinases (MMPs) as quais tem sido demostrado estar aumentadas nas MPS (Baldo et al. 2011) e poderiam estar envolvidas na DRA observada nos pacientes e nos modelos de MPS.

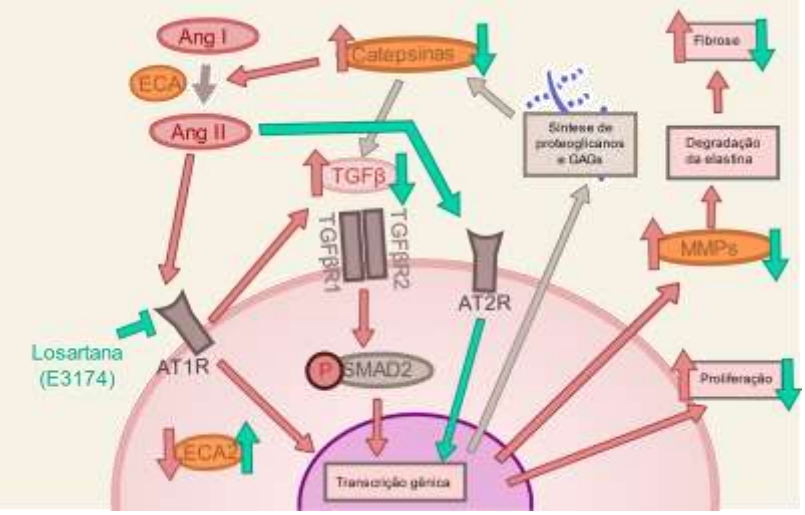

Figura 7. Mecanismos propostos para o papel do sistema renina-angiotensina na fisiopatologia das MPS (setas vermelhas) e o potencial terapêutico da losartana (setas verdes). A conversão de angiotensina I em angiotensina II pode ser potencializada pelo aumento das catepsinas que ocorre nas MPS. A ligação da angiotensina II no receptor AT1, por sua vez, desencadeia o estímulo à produção de TGF-beta e a transcrição gênica com aumento de produção de proteases, degradação da elastina, fibrose e proliferação celular. A via de sinalização do receptor AT1 também estimula a síntese de proteoglicanos e GAGs, o que potencialmente retroalimenta o processo, embora faltem dados sobre essa ativação nas MPS (setas cinzas). O bloqueio do receptor AT1 promovido pela losartana, desvia a angiotensina II para a via do receptor AT2 que desempenha papeis antagônicos, reduzindo a expressão de proteases e a fibrose e promovendo a apoptose. Ang I: Angiotensina I. Ang II: Angiotensina II. AT1R: receptor de angiotensina II tipo I. AT2R: de angiotensina II tipo II. ECA: enzima conversora da angiotensina. ECA2: enzima conversora da angiotensina 2. MMPs: metaloproteinases de matriz.

3.3. Estudos não-clínicos com losartana nas mucopolissacaridoses

Estudos recentes têm apontado que o bloqueio da via Ang II mediante o uso de losartana melhoram alguns dos aspetos clínicos da doença cardiovascular em modelos animais de MPS I (Osborn et al. 2016; Gonzalez et al. 2017). Estes estudos demostram que o tratamento com losartana melhora os parâmetros de fração de encurtamento do ventrículo esquerdo e diminui os diâmetros cardíacos, conduzindo a uma melhora na função cardíaca. Adicionalmente no trabalho de Gonzalez et al (2017), foi demonstrado que os camundongos MPS I tratados com losartana tiveram uma melhora significativa no diâmetro da aorta quando comparados com seus controles. Para além dos efeitos no sistema cardiovascular, um dos trabalhos mostrou resultados positivos também na estrutura óssea dos camundongos tratados, reduzindo as alterações craniofaciais (Osborn et al. 2016). Estes achados apontam que a losartana possa ter um potencial terapêutico para estes órgãos de difícil acesso pelas terapias atuais.

Capítulo 2:
Justificativa

A TRE e o transplante de medula óssea, apesar de serem tratamentos bem estabelecidos, não são capazes de reverter ou impedir a progressão de algumas das manifestações cardiológicas das MPS. Por outro lado, esses pacientes podem se beneficiar de outras formas de tratamento convencionais medicamentosos ou cirúrgicos, os quais podem ser instituídos no momento adequado se houver um melhor conhecimento de como essas manifestações progridem. De modo particular, a ocorrência de DRA, embora descrita em modelos animais, tem sido apenas recentemente avaliada nos estudos sobre as MPS e há uma escassez de dados relacionados à resposta terapêutica das terapias atualmente disponíveis sobre essa alteração em particular. Os dados sobre alterações cardíacas nas diferentes MPS e sobre efeitos da TRE ainda não são completamente conclusivos, e mais casos devem ser analisados.

Além disso, a verificação de um possível efeito da losartana em controlar essas manifestações no modelo animal abre a perspectiva do uso clínico desse medicamento. A losartana é um medicamento de baixo custo e, caso sua segurança seja demonstrada nas MPS, e posteriormente sua eficácia, poderá representar uma terapia acessível e direcionada a necessidades ainda não atendidas desses pacientes.

Capítulo 3:
Objetivos

1. Objetivo Geral

Caracterizar as manifestações cardiovasculares das mucopolissacaridoses e avaliar a segurança da losartana como potencial estratégia terapêutica.

2. Objetivos Específicos

1. Avaliar a progressão da dilatação da raiz de aorta em pacientes com mucopolissacaridoses I, II, IVA e VI.
2. Avaliar a presença de outras manifestações cardiovasculares entre pacientes com MPS I, II, IVA e VI atendidos no Hospital de Clínicas de Porto Alegre.
3. Definir a relação entre o uso de TRE e a progressão das manifestações cardiovasculares.
4. Avaliar a segurança da losartana em pacientes com MPS IVA.
5. Caracterizar os efeitos da losartana no diâmetro da raiz da aorta em pacientes com MPS.
6. Avaliar os efeitos da losartana em marcadores ecocardiográficos de função sisto-diastólica em pacientes com MPS.

Capítulo 4:

Artigo 1 – Aortic root dilatation in patients with Mucopolysaccharidoses and the impact of enzyme replacement therapy

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ORIGINAL ARTICLE

Aortic root dilatation in patients with mucopolysaccharidoses and the impact of enzyme replacement therapy

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Abstract
Mucopolysaccharidoses (MPS) are disorders characterized by impaired glycosaminoglycan (GAG) catabolism as a consequence of a deficiency or the absence of lysosomal enzymes directly involved in their degradation. Multiple organ systems are involved in MPS, including the cardiovascular system. Recently, aortic root dilatation (ARD) has been described in these patients. Thus, we reviewed aortic root diameter measurements in 69 MPS patients from a single center from 2000 to 2016. Aortic root diameter z scores were calculated based on data published by Colan et al. according to the body surface area (BSA) determined using the Haycock formula. The overall incidence of ARD in MPS patients was 39.1%. Higher mean z scores were present in patients with MPS IVA and VI when compared to MPS I and II. Aortic root z scores were higher in older MPS IVA patients, which may suggest a progressive ARD change in this MPS type. No significant differences were found before and after enzyme replacement therapy (ERT) in 11 patients with available data (2 with MPS I; 4 with MPS II; 2 with MPS IVA, and 3 with MPS VI). This work provides further evidence that ARD is common in different types of MPS, being especially evident in MPS IVA, but with a significant occurrence also in MPS VI.

Keywords Aorta · Mucopolysaccharidoses · Enzyme replacement therapy · Dissecting aneurysm

Introduction

Mucopolysaccharidoses are characterized by impaired glycosaminoglycan (GAG) catabolism resulting from a deficiency in lysosomal enzymes directly involved in their degradation. Eleven different MPS disorders are currently described in humans, each with a different enzyme defect. Five main types of GAG accumulation occur in MPS patients depending on the enzyme defect: heparan sulfate, dermatan sulfate, keratan sulfate, chondroitin sulfate, and hyaluronan.

The severity of MPS I and II is well defined; patients with a severe phenotype present cognitive decline. In MPS IV and VI, some patients present more rapid deterioration than others, but cognitive abnormalities are not usually found. Cardiovascular problems are among the main manifestations observed in patients with both severe and attenuated phenotypes, being described in all MPS types except MPS IX. Furthermore, cardiovascular problems, respiratory infections, and restrictive pulmonary disease are among the main causes of mortality [1].

The great vessels may be affected in MPS by thickening of their walls and can be constricted or dilated. Histological abnormalities that are observed in the aortic walls include aortic wall thickening, accumulation of GAGs, and disruption of elastin fibers [2–4]. ARD is frequently found in animal models of MPS I, resulting in the effacement of the sinotubular ridge [2]. Although initial analyses failed to find the same pattern of ARD in humans [2], more recent studies have identified that ARD may also occur in MPS patients, especially those with MPS IVA [5–8].

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Enzyme replacement therapy (ERT) has been available for MPS I, II, and VI since the mid-2000s, and more recently approved for MPS IVA and MPS VII. However, studies in animal models suggest that the recombinant enzyme often fails to reach the aorta and thus has limited impact on this aspect of the disease [9, 10].

Therefore, we aimed to assess ARD occurrence and patterns in a larger sample of patients with different types of MPS collected over the last 16 years. In addition, we assessed the impact of enzyme replacement therapy on the aortic dilatation observed in these diseases.

Materials and methods

Data collection

Following institutional ethical approval, we performed a chart review of patients with MPS that had performed echocardiograms from January 2000 to June 2016. In all cases, we used only the measurement obtained at the sinus of Valsalva (SoV). Aortic root diameter *z* scores adjusted for BSA were calculated as previously described [11] and confirmed using the method of Dallaire et al. [12]. BSA was determined using the Haycock formula [13]. Aortic root dilatation was defined as a *z* score ≥ 2.0.

For comparison between MPS types, only the last echocardiogram of the patients was included in the analysis. All patients with more than one available echocardiogram were included in the analysis of *z* score variation over time.

Patients with available measurements before ERT (up to 5 years before ERT was started) and at least 8 months after that date (up to 5 years after ERT was started) were analyzed for the assessment of pre- and post-treatment *z* scores.

Statistical analysis

All data were entered into PASW Statistics 18.0 for Windows (SPSS Inc., Chicago, IL, USA) and subjected to specific statistical analyses. *Z* score distribution was assessed with the Shapiro–Wilk and Kolmogorov–Smirnov tests, and as normal distributions were observed, parametrical tests were used in all subsequent analyses. Mean *z* score comparisons between MPS groups were calculated using ANOVA with Tukey's post hoc test. Linear regression and Pearson's correlation coefficient were used to assess *z* score variation over time. *Z* score variation over time for individual patients with each type of MPS was assessed using a mixed model analysis corrected for treatment status (with or without ERT), including all patients with more than one available echocardiogram. A paired *t* test was used to compare pre- and post-treatment *z* scores. Bonferroni correction was used for multiple comparisons. A p value ≤ 0.05 was considered significant.

Results

A total of 69 patients with MPS were included (25 with MPS I, 21 with MPS II, 16 with MPS IVA, and 7 with MPS VI) (Table 1). In total, 27 of the 69 patients (39.1%) met the criteria for ARD. Higher mean *z* scores were present in patients with MPS IVA and VI when compared to MPS I and II (Table 1). ARD was found more frequently in the former group. Among patients with MPS I, 5 (20.0%) met the criteria for ARD, and 6 (28.6%), 11 (68.8%), and 5 (71.4%) for MPS II, MPS IVA and MPS VI, respectively (Fig. 1). Moreover, patients with the severe MPS I phenotype had higher *z* scores when compared to those with an attenuated phenotype (Fig. 2).

Aortic root *z* scores were higher in older MPS IVA patients, which may suggest that ARD is progressive in

Table 1 Demographic characteristics and aortic root measurements among MPS patients

	Overall ($N=69$)	MPS I ($n=25$)	MPS II ($n=21$)	MPS IVA ($n=16$)	MPS VI ($n=7$)	p
Age (years)	14.4 (9.9)	14.6 (12.1)	13.0 (9.1)	15.8 (8.9)	15.0 (5.4)	0.852
Weight (kg)	30.5 (16)	30.9 (19.3)	35 (16.6)	25.6 (9.3)	27.1 (11.8)	0.333
Height (cm)	114.7 (21.1)	117.5 (27.3)	122.3 (17.4)	102.2 (9.7)	110 (11.3)	0.023[a]
BSA (m^2)	1 (0.3)	1 (0.4)	1.1 (0.3)	0.9 (0.2)	0.9 (0.2)	0.206
SoV (cm)	2.3 (0.4)	2.2 (0.4)	2.3 (0.4)	2.5 (0.4)	2.3 (0.4)	0.137
z score	1.7 (1.6)	1.1 (1.6)	1.2 (1.4)	3.1 (1.5)	2.1 (1.1)	0.001[b]

Values are mean (±SD)

BSA body surface area. *SoV* aortic root diameter at the sinus of Valsalva

[a]MPS IVA different from the other groups

[b]MPS IVA and VI are different from MPS I and II

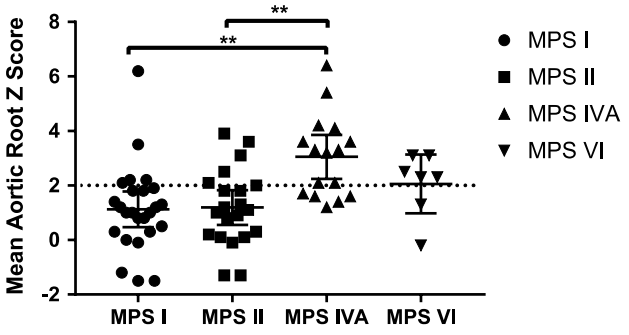

Fig. 1 Mean aortic root diameter in different MPS types. Only the final measurement was included in this analysis. $^{**}p < 0.01$. ANOVA and Tukey's post hoc

this type of MPS (Fig. 3c). The mean MPS IVA *z* score increase was 0.058 per year (95% CI 0.004–0.113). A positive linear regression slope was also observed in the MPS VI group, but the correlation was not statistically significant (Fig. 3d).

An inverse correlation was observed between aortic root *z* scores and age in MPS I and MPS II (Fig. 3a, b), but this may be due to the presence of attenuated phenotypes in the older patients. When only including patients with severe phenotypes, we did not observe a significant change with age, which is possibly due to the small number of patients (data not shown).

Pre- and post-ERT *z* score data were available for 11 patients (2 with MPS I; 4 with MPS II; 2 with MPS IVA, and 3 with MPS VI). There was no statistically significant variation in the *z* scores between the two periods ($p = 0.697$; Figs. 4a–d). For all of the presented analyses, comparable results were obtained when using the Dellaire et al. [12] method to calculate *z* scores (data not shown).

Discussion

In this study, we evaluated aortic root diameter in 69 patients with MPS, which represents the largest collection of MPS patients to date. Our results confirm previous reports showing a significant proportion of MPS patients with ARD (39.1% in this study; 35.3% of 36 patients in a previous report [8]). In addition, we report for the first time that a large proportion of MPS VI patients have aortic dilatation. Considering that MPS are progressive disorders, aortic pathology is also expected to be progressive. However, variation in aortic root diameter over time was not observed in the mixed models analysis for any of the MPS types (data not shown). This may reflect either a slow progression rate, which could not be measured in our sample, or the fact that aortic root diameter ceases to dilate beyond a certain point. Supporting the former hypothesis, aortic root *z* scores were higher in older patients, at least in MPS IVA. This finding is consistent with a previous report identifying a correlation between age and aortic root dimensions in MPS IVA [6].

We confirmed that ARD is found predominantly in patients with the severe MPS I phenotype (Hurler syndrome) [5], which may explain the inverse correlation between age and *z* scores in these patients. Hurler syndrome is characterized by developmental delay/intellectual disability. Somatic manifestations are also more severe in these patients, especially when compared to the most attenuated phenotype (Scheie syndrome). Some patients are situated in the middle of the spectrum with more prominent somatic involvement and little-to-no intellectual dysfunction, which is classified as Hurler–Scheie syndrome. A binary classification of severe (Hurler syndrome) and attenuated (Hurler–Scheie and Scheie syndromes) phenotypes is also used. As patients with the severe phenotype have a lower life expectancy, older MPS I patients are mostly represented by those with the

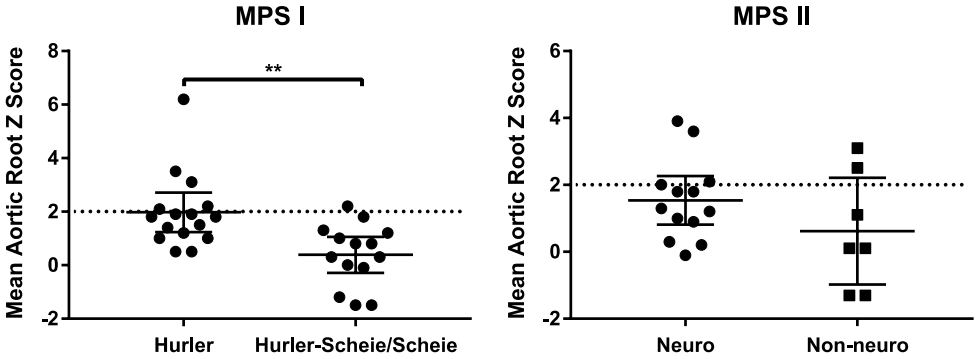

Fig. 2 Aortic diameter according to disease severity. Mean aortic root diameter is higher in MPS I patients with Hurler (severe) than Hurler–Scheie and Scheie (attenuated) phenotypes. In MPS II, there is no significant difference in aortic root diameter between neuronopathic and non-neuronopathic patients. Only the final measurement was included in this analysis. $^{*}p < 0.05$, Student's *t* test

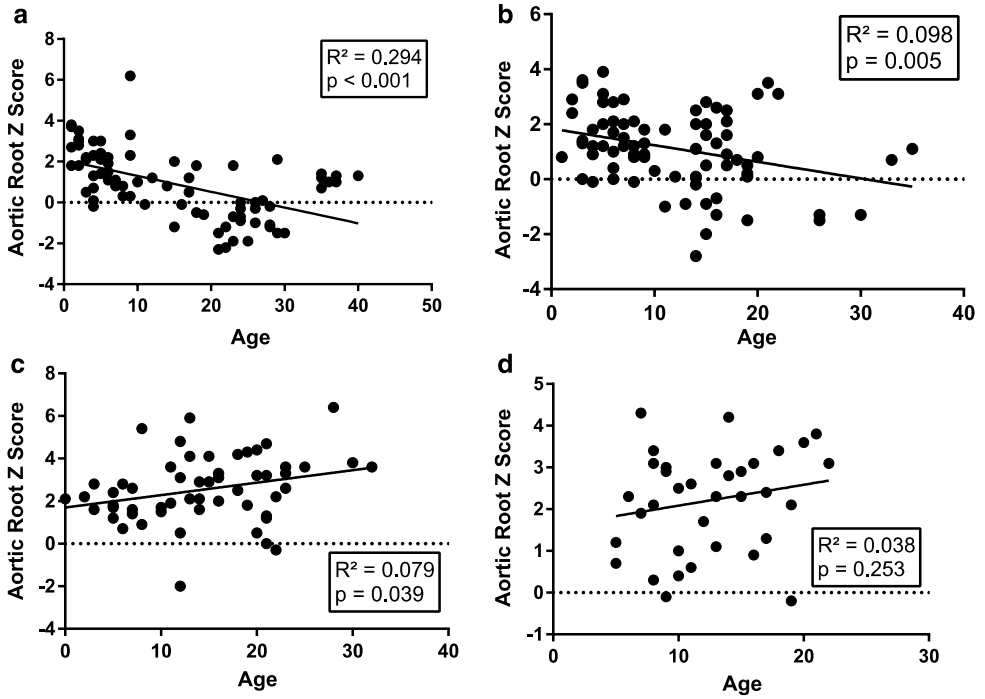

Fig. 3 Aortic root dilation versus age. Aortic root diameter *z* score values at different ages in MPS I (**a**), MPS II (**b**), MPS IVA (**c**), and MPS VI (**d**). Multiple measures were obtained from the same patients at different ages when available

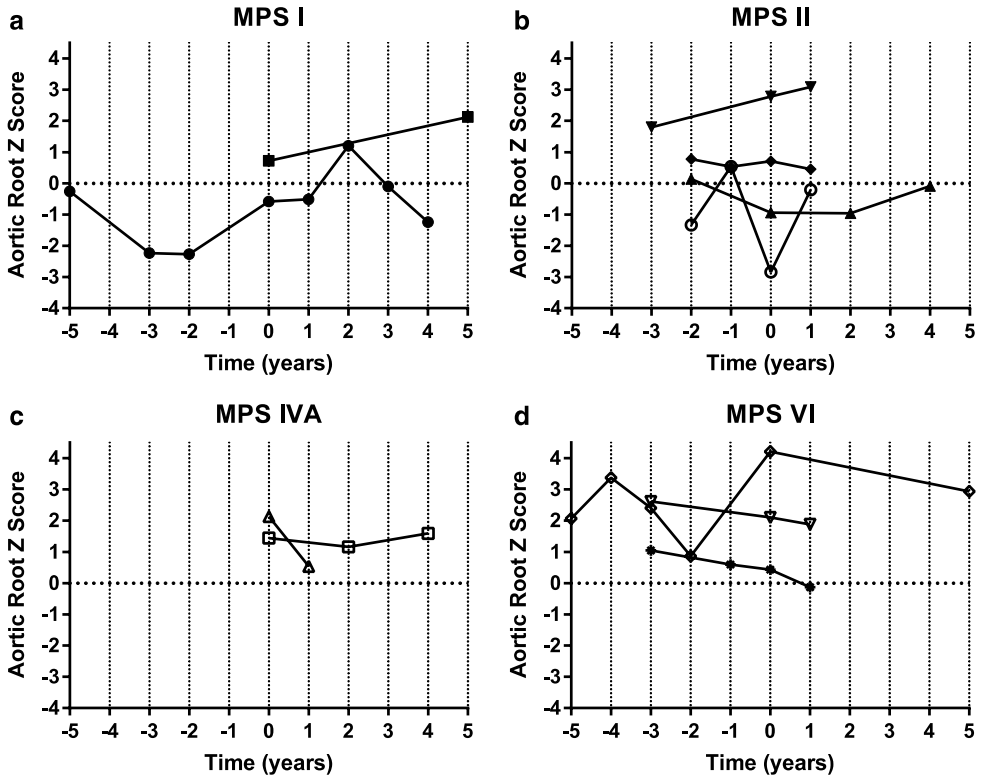

Fig. 4 Impact of ERT on aortic root diameter. Aortic root diameter *z* scores for single patients before and after ERT in MPS I (**a**), MPS II (**b**), MPS IVA (**c**), and MPS VI (**d**)

attenuated phenotype who would also be expected to have a less prominent involvement of the aorta [14].

No significant association was observed between aortic root diameter and disease severity in MPS II patients. A similar pattern was found along the severity spectrum (Fig. 2). Similar to MPS I, the classification of the disease in MPS II depends on the presence (neuronopathic form) or absence (non-neuronopathic form) of intellectual disability. Furthermore, as in MPS I, patients with the neuronopathic form have more severe somatic involvement. In fact, increased somatic burden and decreased neurocognitive ability were shown to be correlated even in patients with attenuated MPS I and MPS II phenotypes [15]. Thus, it is reasonable to believe that the same association identified in MPS I is also present in MPS II patients, although this could not be clearly demonstrated herein.

ARD identification has clinical importance, as it may progress to aortic dissection [16]. Aortic dissection generally occurs in older patients (mean age 62 years), although it may occur earlier in high-risk groups, including patients with Marfan or Turner syndromes [17, 18]. Although MPS patients have not been shown to be at increased risk for this uncommon life-threatening condition, it is known that the GAG content increases in dissecting aneurysms of the thoracic aorta in humans [19, 20].

Currently, elective aortic surgery is indicated for adult patients with an aortic root diameter ≥ 55 mm with the objective of preventing aortic dissection [21]. In Marfan syndrome, the threshold for this indication is 50 mm, and in the presence of additional risk factors (i.e., family history of dissection, progressive dilatation > 2 mm/year, severe aortic or mitral regurgitation, and desire for pregnancy) it is reduced to 46 mm [22]. While recommendations for surgery based on z score values are lacking, it is generally assumed that a value < 4.0 does not represent an imminent risk for aortic dissection [18]. In their cohort, Bolourchi et al. [8] did not observe any case of aortic dissection. This complication was also not observed in this study and only one MPS I and four MPS IVA patients had z scores > 4.0 (Fig. 1). None of our patients had an absolute aortic root dimension greater than 45 mm.

We did not observe any effect of ERT on aortic root measurements. This is in accordance with findings of other authors [6, 8]. However, in the case of MPS IVA, this evaluation can be especially difficult because ERT was only recently approved [6]. While it is still unclear if ERT can prevent or treat ARD in MPS patients, studies in mouse models demonstrate that this abnormality can be responsive to losartan [23]. The same medication also promotes improvements in left ventricle diameter, shortening, fraction, and craniofacial aspects in animal models [23, 24].

This work has some limitations. Although this is the largest cohort of MPS patients reported to-date, the aortic root measurement was not performed in the controlled environment of a clinical trial due to retrospective data being used. Furthermore, for a single patient, different echocardiographers were involved during the follow-up period, which may have affected the results. Regardless, our results are consistent with the literature. Another limitation is that we relied on echocardiography data to obtain the aortic root measurements (as other published studies in patients with MPS have). The use of more precise methods, such as cardiac MRI, may allow more robust analyses of the entire thoracic aorta. Nevertheless, risks related to sedation in young children should be considered. Finally, using z score parameters in short-stature patients may likely overestimate the parameter, pointing to the existence of aortic dilatation in a setting where the growth of the aorta is similar to age-matched individuals, although disproportional to the patient's height and thorax dimensions. In Turner syndrome, a condition in which both aortic root dilatation and short stature are observed, a disease-specific parameter was recently developed [25]. As there is no such parameter for MPS, we managed to reduce this bias using an additional multivariate formula recently published and used for Turner syndrome [12, 26]. The results were unaffected.

In summary, this work supports evidence that ARD is a clinical manifestation of different types of MPS. It also confirms that this finding is particularly prominent in MPS IVA patients. Moreover, it identifies a significant involvement in patients with MPS VI for the first time. ERT did not reduce aortic diameters, and ancillary therapies, such as the use of losartan, may be needed for MPS patients with aortic dilatation.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent For this type of study, formal informed consent is not required.

Research involving human and/or animal participants This article does not contain any animal studies performed by any of the authors.

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Capítulo 5:

Artigo 2 – Cardiac Disease in Patients with Mucopolysaccharidoses and the Impact of Enzyme Replacement Therapy

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Artigo submetido à revista *Journal of Inherited Metabolic Disease*.

Original Research Article

Cardiovascular Manifestations in Patients with Mucopolysaccharidoses

Poswar et al. Cardiac Manifestations in Mucopolysaccharidoses.

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Abstract

Background: Cardiovascular involvement is among the main features of the MPS disorders and it is also a significant cause of morbidity and mortality. The range of manifestations include cardiac valve disease, conduction abnormalities, left ventricular hypertrophy and coronary artery disease. Here, we assessed the cardiovascular

manifestations in a cohort of patients with MPS, as well as the impact of enzyme replacement therapy (ERT) on those manifestations.

Methods: We performed chart review of 76 patients with different types of MPS that had performed echocardiograms from January 2000 until October 2018. Standardized Z scores were obtained for heart chamber sizes according to the body surface area. When available, echocardiographic measurements that were performed before ERT and at least 18 months after that date were used for the assessment of pre- and post-treatment measurements.

Results: Left side valvular disease was a frequent finding, with mitral and aortic thickening being reported in most patients in all four MPS types. Left atrium dilatation was present in 26% of the patients; 8% had increased left ventricular mass; 28% had pulmonary hypertension and only one patient had reduced ejection fraction. ERT had a significant impact in left ventricular hypertrophy parameters, but failed to improve valve abnormalities, pulmonary hypertension and left atrial dilatation.

Conclusions: Our results confirm the impact of long-term ERT on left ventricular hypertrophy and its limitations in reversing other prevalent cardiovascular manifestations.

Keywords: Mucopolysaccharidoses; Enzyme replacement therapy; Pulmonary Hypertension; Left Ventricular Hypertrophy; Left Atrium; Heart Valve Disease.

Introduction

The mucopolysaccharidoses (MPS) are a group of eleven disorders characterized by an impaired catabolism of glycosaminoglycans (GAGs) as a consequence of a deficiency of lysosomal enzymes directly involved in their degradation, resulting in the accumulation of one or more of five different types of GAGs: heparan sulfate, dermatan sulfate, keratan sulfate, chondroitin sulfate and hyaluronan [1]. According to the accumulated substrate and clinical features, the MPS disorders are classified in seven main types (I, II, III, IV, VI, VII, and IX), with MPS III and MPS IV being further subclassified in four (IIIA, IIIB, IIIC and IIID) and two types (IVA and IVB), respectively, according to the enzymatic defect [2].

Cardiovascular involvement is among the main features of the MPS disorders and it is also a significant cause of morbidity and mortality [3]. As GAGs are a significant normal component of cardiac structures, enzymatic deficiencies related to GAG degradation results in prominent storage of undegraded GAGs in heart structures, which may cause tissue damage through the activation of cell proteases [3,4]. This results in many different manifestations, including cardiac valve disease, conduction abnormalities, hypertrophy of the left ventricle and coronary artery disease[3].

Replacing the deficient enzyme, either through enzyme replacement therapy (ERT) or Hematopoietic Stem Cell transplantation (HSCT), is the current paradigm of targeting the primary defect in MPS. HSCT is widely used in severe presentations of MPS I and ERT is available for the treatment of MPS types I, II, IVA, VI and VII [5]. Nevertheless, real-world experience with those disease-modifying therapies has unveiled several limitations, especially regarding their ability to reverse or even halt the progression of some cardiovascular manifestations in MPS patients [5,6].

In this study, we aimed to assess the cardiovascular manifestations in a cohort of patients with different MPS types, as well as the impact of enzyme replacement therapy on those manifestations.

Methods

Data collection

Following institutional ethical approval (17-0013; Grupo de Pesquisa e Pós Graduação, Hospital de Clínicas de Porto Alegre, Porto Alegre, Brazil), we performed chart review of patients with MPS that had performed echocardiograms from January 2000 until October 2018. When available, electrocardiographic data was also recorded.

Body surface area was calculated using the geometric method of Haycock [7]. Left ventricular mass (LVM) was obtained using the formula of Devereaux [8]. Standardized Z scores for left ventricle posterior wall thickness (LVPWT) and interventricular septum thickness (IVST) were calculated using the methods described by Lopez et al. [9], while, for the left ventricular mass (LVM) and left atrium parasternal long axis anteroposterior dimension (LAD) an online resource from Boston Children's Hospital Heart Center was used [10,11]. Relative wall thickness was obtained using the formula (2 × LVPWT)/(LV internal diameter at end-diastole) and a cut-off value of 0.41 was used for classification of the left ventricle geometry, as previously described [12]. Left ventricle ejection fraction (LVEF) was obtained through the method of Teichholz[13] and systolic pulmonary artery pressure was obtained with doppler flow studies. For the analysis of the prevalence of the cardiac manifestations, the last available echocardiogram was used. When available, echocardiographic measurements performed before ERT (up to 18 months before ERT was

started) and at least 18 months after that date (up to 166 months after ERT was started) were used for the assessment of pre- and post-treatment measurements. Those patients who received ERT in the peri-transplant period or as a concomitant therapy after transplantation were not included in that assessment.

For those who also had an available electrocardiogram, the presence or absence of left atrial enlargement, repolarization disorder and left ventricular hypertrophy according to the clinical report were recorded. Interval measurements and Z scores for QRS duration [14] were also obtained. Corrected QT (QTc) interval was calculated using the Bazett formula[15].

Statistical analysis

All data was entered into PASW Statistics 18.0 for Windows (SPSS Inc., Chicago, IL, USA) and submitted to specific statistical analysis. Graphics and part of the statistic tests were generated using the GraphPad Prism version 7.0. Normality of the samples was assessed with Shapiro-Wilk and D'Agostino & Pearson tests. Nonparametric Kruskal-Wallis and Dunn's post-hoc tests were used for the comparison of quantitative measurements among different MPS types. Chi-squared test was used for comparison of the frequencies of abnormalities among MPS types. For the comparison between baseline and follow-up parameters after ERT, the parametric paired t-test was used to assess Z-scores of cardiac structures and the Wilcoxon matched-pairs signed rank test was used to assess cardiac valves. A p-value of less than 0.05 was considered significant.

Results

A total of 76 patients (27 MPS I, 22 MPS II, 19 MPS IVA and 8 MPS VI) were included in this study. Most of those patients were analyzed in a previous publication, which focused on the aortic root dimension [6]. All MPS II patients were males. MPS IVA patients had a lower median height, when compared to MPS II patients and were less frequently treated with ERT, when compared to MPS VI patients (table 1).

Valvular disease

Among the 76 patients included in this study, left side valvular disease was a frequent finding, with mitral and aortic thickening being reported in most patients in all four MPS types (Figure 1). Furthermore, mitral and aortic insufficiency, mostly mild, were frequently found in patients with MPS I, II and VI, but were also observed in a significant proportion of patients with MPS IVA. To a lesser extent, tricuspid valve thickening and insufficiency were also present in patients with MPS types I, II and VI. Involvement of the pulmonary valve was observed in a few patients with MPS I and II.

Other echocardiographic measurements.

Left ventricular hypertrophy parameters, including LVPWT, IVST Z scores and RWT, were above average in a significant proportion of patients in all subgroups (Figure 3 and supplementary figure 2). Nevertheless, LVM Z scores were normal in the last available echocardiogram of most of the patients (supplementary table 1).

When assessed the last available echocardiogram, the median left atrium diameter (LAD) and the estimated systolic pulmonary artery pressure (SPAP) were increased in all groups (figure 3D and 3E). A total of 26% and 28% of the patients had LAD or SPAP above normal reference limits, respectively (supplementary table 1). The median value of SPAP

was significantly lower in MPS IVA than in MPS I (figure 3D). Left ventricle ejection fraction was preserved in all, but one patient with MPS IVA, who also had eccentric hypertrophy, severe aortic insufficiency and moderate aortic stenosis (figures 2A, 2B and 3F).

Effects of the enzyme replacement therapy

For those 19 patients where echocardiographic measurements were available before and after ERT start, ERT did not lead to significant improvements in valvular disease (supplementary figure 1). However, significant reduction of left ventricular hypertrophy parameters (including IVST, LVPWT, LVM and RWT) was observed after ERT was started (Figure 3G-I and supplementary figure 2). No statistically significant change in SPAP, LAD or LVEF were observed after ERT (figure 3J-L).

Electrocardiogram

Electrocardiograms were performed in 66 patients. All patients had a sinus rhythm, although transient junctional rhythm was observed for one patient with MPS IVA. The most frequently observed abnormality was the electrocardiographic criteria for left ventricular hypertrophy (27%) (table 3 and figure 4A). Repolarization anomalies (figure 4A) and left atrium hypertrophy (figure 4B) were present in 6% of the patients. Increased PR and QTc intervals were also occasionally observed (figure 4C).

Discussion

In this study, we assessed the prevalence of cardiac manifestations in a cohort of patients with MPS I, II, IVA and VI; which included mostly patients treated with ERT. In

agreement to previous reports [3,16–19], valve involvement comprised mainly left sided valves and affected a high proportion of patients. Mitral valve involvement was more common than aortic valve in the four MPS types. We could not identify any significant worsening or improvement of valve pathology after ERT. It is widely accepted that ERT has limited impact in valve abnormalities of patients with MPS [12,20–24], probably due to poor tissue penetration and irreversibility of the valvar damage. Nevertheless, it may have a role of preventing or delaying its appearance when treatment is started very early, as suggested by studies with sibling pairs and animal models [25,26].

The finding of normal Z scores for LVM in a significant proportion of the patients in our cohort may reflect the effects of long-term therapy in this parameter. Accordingly, significant reductions of IVST, LVPWT and LVM were observed in the follow-up measurements, which is in agreement to prior studies and generally acknowledged as a well-stablished effect of ERT [3,5,12,21–23].

We found a high proportion of patients with left atrial diameter Z scores above normal limits (26%), but a lower prevalence of left atrial enlargement criteria on electrocardiogram (6%). This difference may be attributed to a lower sensitivity of the latter method as an indicator of left atrial enlargement[27]. Previous studies have reported the presence of left atrial dilatation in 6.3% of patients with MPS IVA [28] and 2.3% of patients with MPS VI [12]. Another study reported 10.7% of MPS patients with biatrial enlargement criteria on electrocardiogram [24]. As there is a higher proportion of aortic root dilatation in patients with MPS [6,29], the use of left atrium to aortic root ratio may underestimate true prevalence of atrial enlargement [30]. The impact of ERT in left atrial dimension is not stablished.

Systolic pulmonary artery pressure (SPAP) was increased in 28% of the patients. Other authors have reported a prevalence of 36% of pulmonary hypertension (PH) in a sample of 28 pediatric patients and emphasized that it was the main cause of death in their cohort [16]. Chronic hypoxemia secondary to obstructive sleep apnea is a known cause of pulmonary hypertension among MPS patients, but it cannot explain all cases, and a role for the left heart dysfunction in its pathogenesis is also likely [16,31,32].

When analyzing the whole sample, we could not demonstrate a statistically significant improvement or worsening of the left ventricle ejection fraction. Nevertheless, ejection fraction was already preserved in all but one patient with MPS II. Also, the MPS IVA patient reported with low LVEF in figure 3F and supplementary table 1 has never received ERT. In the literature, the impact of ERT in ejection fraction is less clear, with some studies pointing to stabilization or reduction of the mean value over time [12,33] and others showing improvements in those patients who already had reduced LVEF on baseline [34]. Although those inconsistences need further clarification, it is possible that ERT may improve LVEF in a subset of patients with prior systolic dysfunction, while not being able to prevent a slow long-term deterioration.

In our study, all subjects had a sinus rhythm and none was on pacemakers. However, it is likely that the prevalence of cardiac conduction abnormalities may increase strikingly in older MPS patients [35]. Furthermore, although most MPS patients will not have a clinically significant conduction abnormality, resting electrocardiogram and 24h Holter monitoring, when indicated, should be integral part of the care of MPS patients, considering that the early recognition may have a high impact in morbidity and mortality [35].

This work has some limitations. The measurements were performed by more than one echocardiographer and the time frame of the study involved a period before the establishment of new guidelines for echocardiographic measurements in our center, when newer quantification methods for LA size, ejection fraction and LVM were included in the routine echocardiogram protocol. Nevertheless, by including data from a wide period of time, we were able to estimate the prevalence of cardiovascular abnormalities in a large sample of MPS patients as well as the long-term effectiveness of ERT.

In summary, we identified a high proportion of MPS patients with cardiac abnormalities, which ranged from isolated mitral or aortic thickening to a more severe clinical picture including moderate to severe valvular insufficiency or stenosis, left ventricular hypertrophy, pulmonary hypertension and, rarely, heart failure with reduced ejection fraction. We also confirmed the impact of long-term ERT on left ventricular hypertrophy in the mucopolysaccharidoses and its limitations in reversing other cardiovascular manifestations, such as the valvular involvement.

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Disclosures:

None.

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Figures

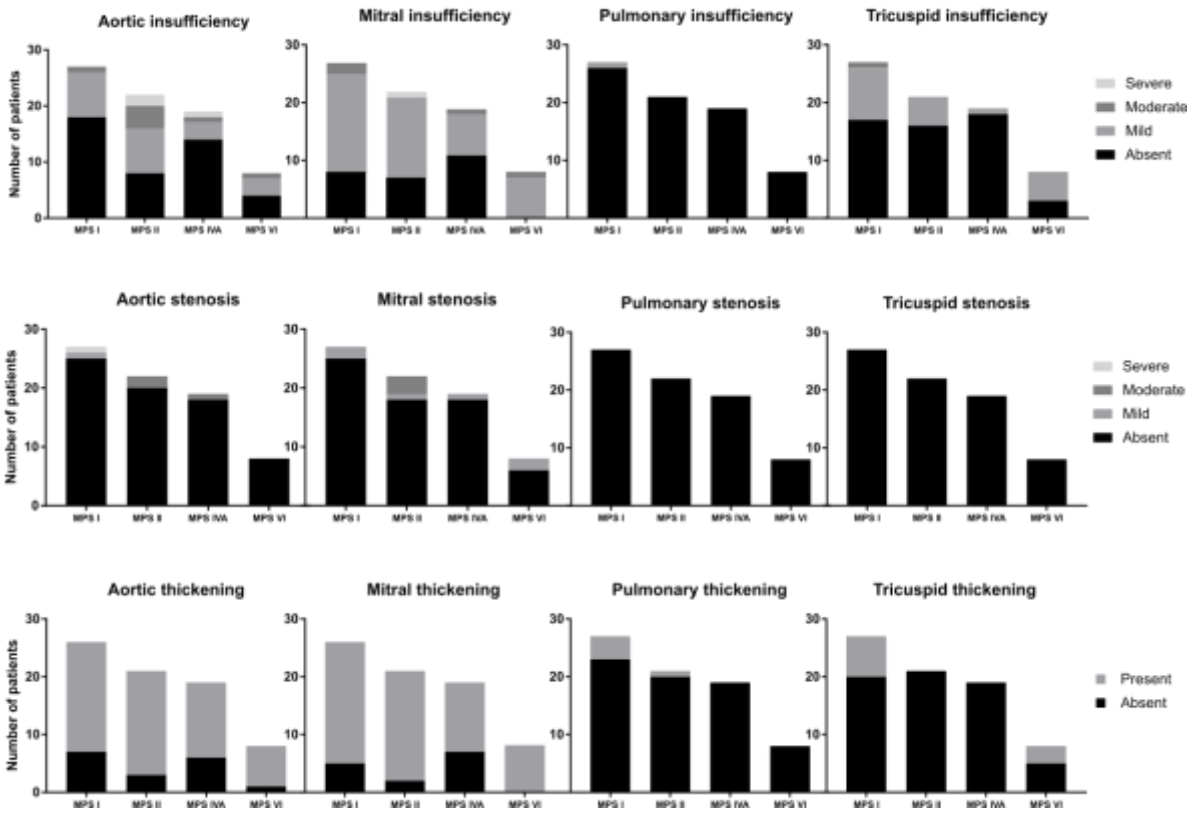


Figure 1. Prevalence of heart valve abnormalities in MPS types I, II, IVA and VI, including both treated and untreated subjects.

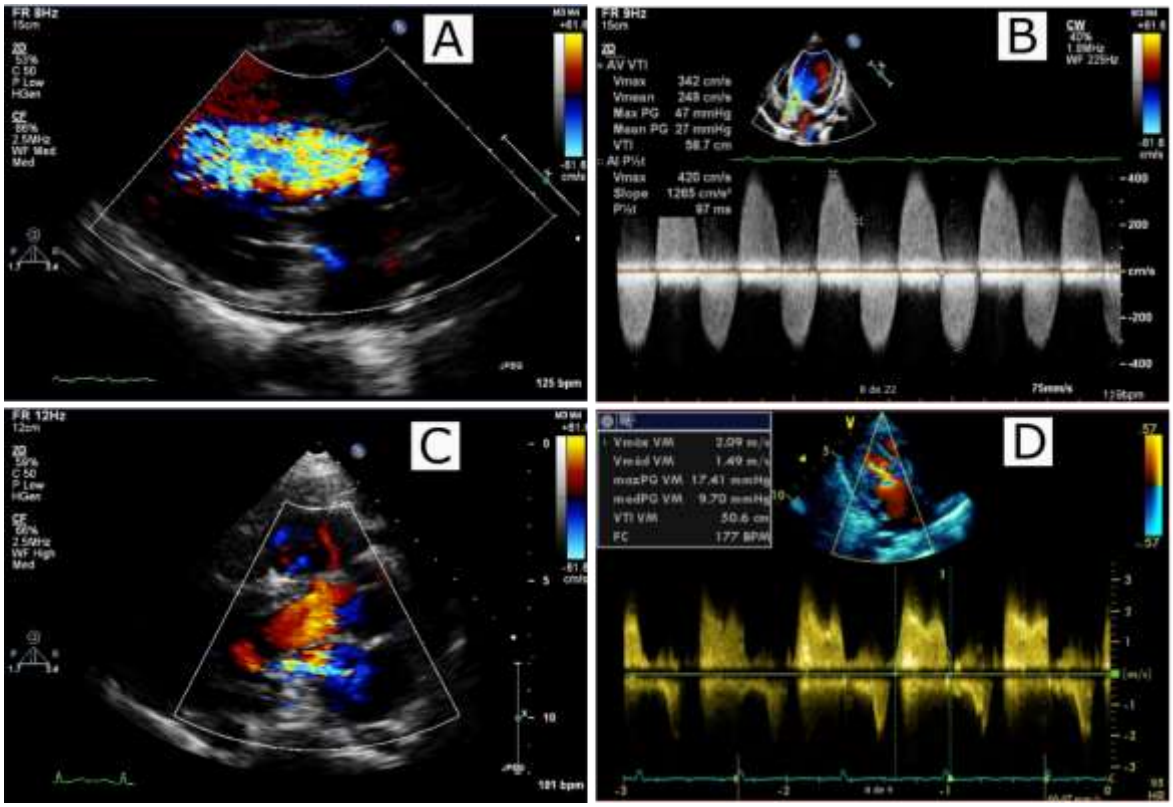


Figure 2. Representative abnormalities identified in the valves of the patients of this study. Aortic insufficiency (A) and aortic stenosis (B) in a 28-year-old female with MPS IVA, who also had a reduced ejection fraction (35%). Mitral insufficiency (C) in a 24-year-old male with MPS VI. Mitral stenosis (D) in a 17-year-old male with MPS II.

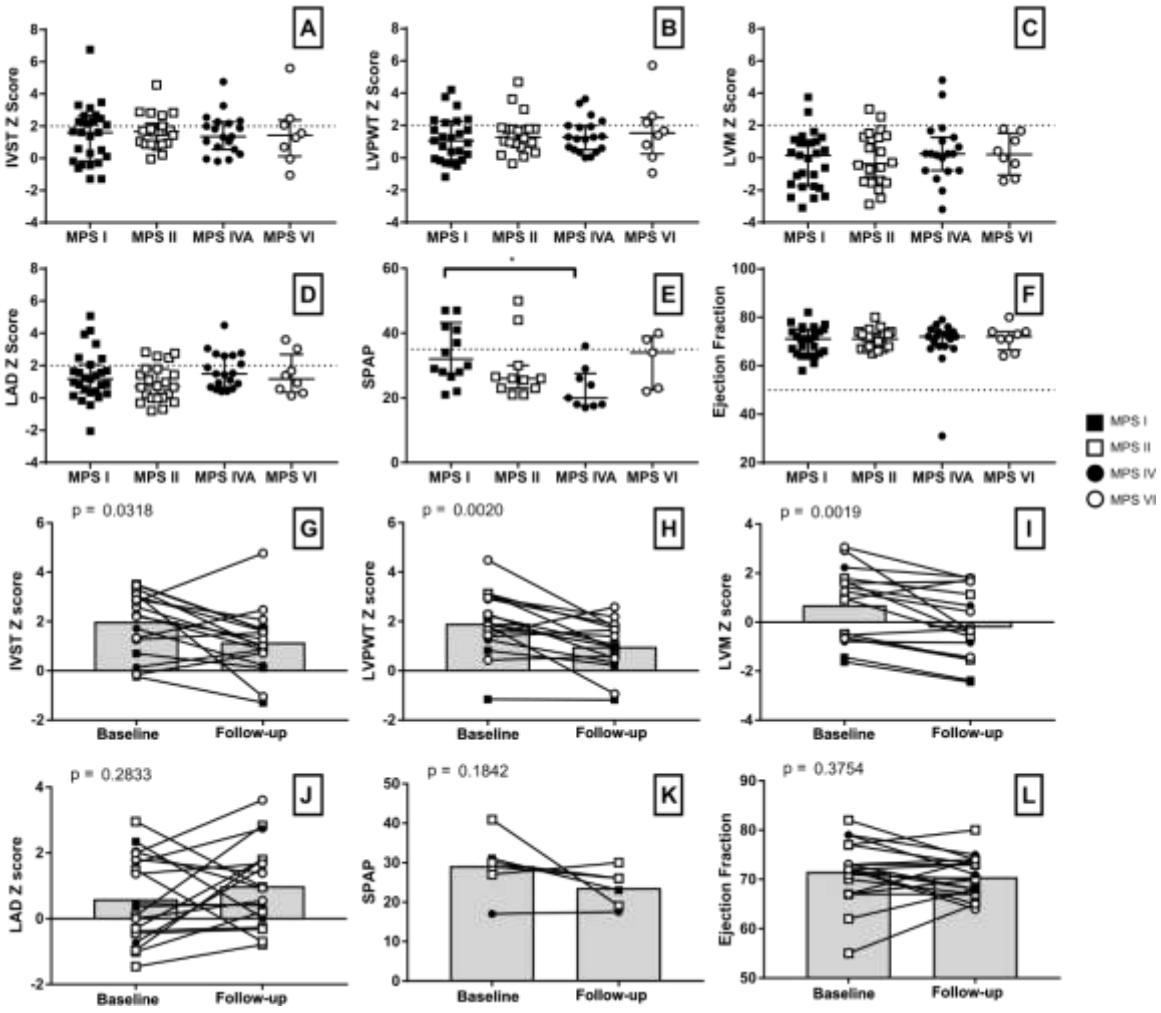


Figure 3. Comparison of measurements of echocardiographic parameters among MPS types (A-F) as well as before and after ERT for single individuals (G-L), including the Z scores of interventricular septum thickness (A, G), left ventricle posterior wall thickness (B, H), left ventricle mass (C, I), left atrium diameter (D, J), systolic pulmonary artery pressure (E, K) and ejection fraction (F, L). A 10-year-old girl with severe MPS I fell outside the axis of both IVST (A) and LVPWT, with Z scores values of 9.0 and 9.7, respectively. Another MPS I patient (a 29-year-old female with Hurler-Scheie phenotype) fell outside the axis range of SPAP (E), because she had an SPAP of 110 mmHg. Statistical analysis with Kruskal-Wallis and Dunn's post-hoc test for comparisons among MPS types

(A-F) and with paired t test for before and after analyses (G-L). * $p < 0.05$. MPS IVA is different from MPS I.

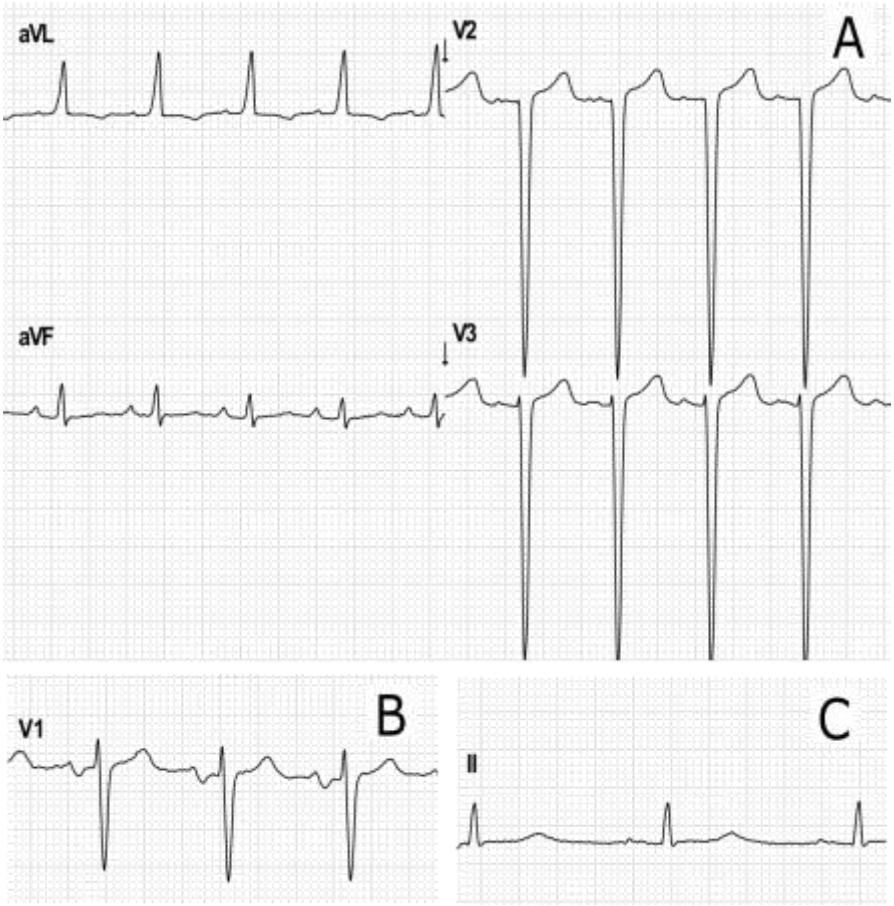


Figure 4. Representative electrocardiographic abnormalities identified in the patients of this study. A: Electrocardiographic criteria for left ventricular hypertrophy (Cornell index: 40 mm) and repolarization abnormalities in a 28-year-old female with MPS IVA. B: Left atrium enlargement in a 24-year-old male with MPS II (terminal negative deflection within the P wave in lead V1). C: Increased PR interval (203 ms) and transient prolonged QTc (464 ms) in a 35-year-old female with MPS I during postoperative care for aortic valve and mitral valve replacement.

Tables

Table 1. Characteristics of the subjects

	Overall (n=76)	MPS I (n=27)	MPS II (n=22)	MPS IVA (n=19)	MPS VI (n=8)	*p*-value
Sex (n)	M: 46 F: 30	M: 12 F: 15	M: 22 F: 0	M: 8 F: 11	M: 4 F: 4	**<0.001**†
Age (years)	11.3 (6.4–19.1)	10.2 (5.3–25.7)	11.8 (8.1–23.6)	10.1 (6.8–15.3)	16.2 (10.9–19.2)	0.491
Weight (kg)	25.6 (19.0–40.0)	23.5 (16.1–39.5)	28.0 (25.5–62.0)	21.0 (16.3–32.9)	22.3 (17.4–32.3)	0.103
Height (cm)	111.0 (100.0–135.0)	108.0 (94.0–142.4)	129.0 (115.0–151.0)	99.5 (89.1–108.8)	106.5 (102.1–120.5)	**0.003**‡
BSA (m^2)	0.9 (0.7–1.2)	0.9 (0.7–1.3)	1.0 (0.9–1.6)	0.8 (0.7–1.0)	0.8 (0.7–1.1)	0.057
Treated with ERT (n)	47/76	16/27*	15/22	8/19	8/8	**0.029**§
Time on ERT (months)	41.0 (18.0–95.0)	52.0 (17.3–96.8)	64.0 (13.0–89.0)	22.5 (16.5–38.8)	73.5 (26.8–105.8)	0.317

Continuous variables are reported as median and interquartile range. *4 patients who received ERT as a concomitant therapy for HSCT are not included in the ERT-treated group. Statistical analysis with chi-squared and partitioning for categorical variables and Kruskal-Wallis test with Dunn's post-hoc test for continuous variables. † MPS II is different from other MPS types; ‡. MPS IVA is different from MPS II; §. MPS IVA is different from MPS VI.

Table 2. Parameters of the last available resting electrocardiogram, including both ERT treated and untreated subjects.

	Overall (n=66)	MPS I (n=20)	MPS II (n=19)	MPS IV (n=19)	MPS VI (n=7)	*p*-value
Heart Rate (bpm)	99 (80–107)	92 (77–106)	93 (72–106)	103 (91–108)	99 (85–107)	0.527
PR interval (ms)†	149 (138–168)	148 (138–156)	169 (142–178)	146 (138–151)	166 (151–181)	0.168
QRS interval (ms) †	85 (80–97)	82 (80–91)	90 (83–100)	81 (79–94)	82 (78–86)	0.627
QRS interval Z score†	0.0 (-0.6–1.0)	0.2 (-0.3–1.0)	-0.1 (-0.3–0.6)	-0.1 (-0.8–1.0)	-1.0 (-1.3–-0.7)	0.208
Prolonged QRS (Z > 2.0)†	2/41 (5%)	0/11 (0%)	1/12 (8%)	1/16 (6%)	0/2 (0%)	0.789
QTc interval (ms) †	385 (374–394)	387 (380–394)	389 (366–399)	382 (375–393)	367 (358–376)	0.529
QTc > 450 ms†	1/41 (2%)	1/11 (9%)	0/12 (0%)	0/16 (0%)	0/2 (0%)	0.424
QRS wave axis (°)†	60 (29–76)	76 (59–91)	48 (27–74)	37 (15–73)	82 (62–101)	**0.027** [a]
Left atrial enlargement	4/66 (6%)	2/20 (10%)	2/19 (11%)	0/19 (0%)	0/8 (0%)	0.387
Repolarization disorder	4/66 (6%)	1/20 (5%)	1/19 (5%)	2/19 (11%)	0/8 (0%)	0.752
Left ventricular hypertrophy	18/66 (27%)	4/20 (20%)	6/19 (32%)	7/19 (37%)	1/8 (13%)	0.517

Continuous variables are reported as median and interquartile range. † Measurements performed for the 41 patients with available ECG tracings. Statistical analysis with chi-squared for categorical variables and Kruskal-Wallis test with Dunn's post-hoc test for

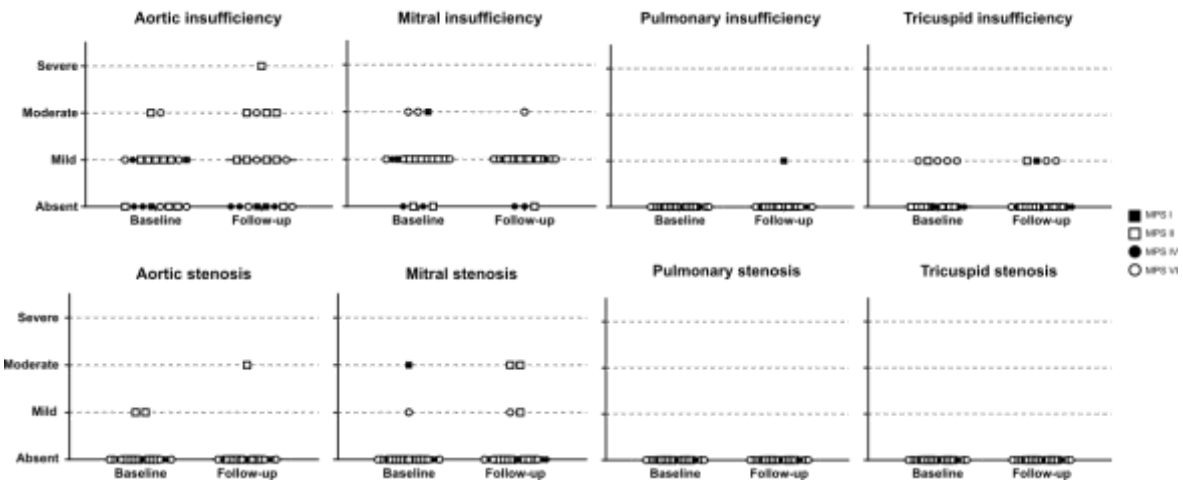
continuous variables. a. $p<0.05$, but no statistically significant differences in pairwise comparisons.

SUPPLEMENTAL MATERIAL

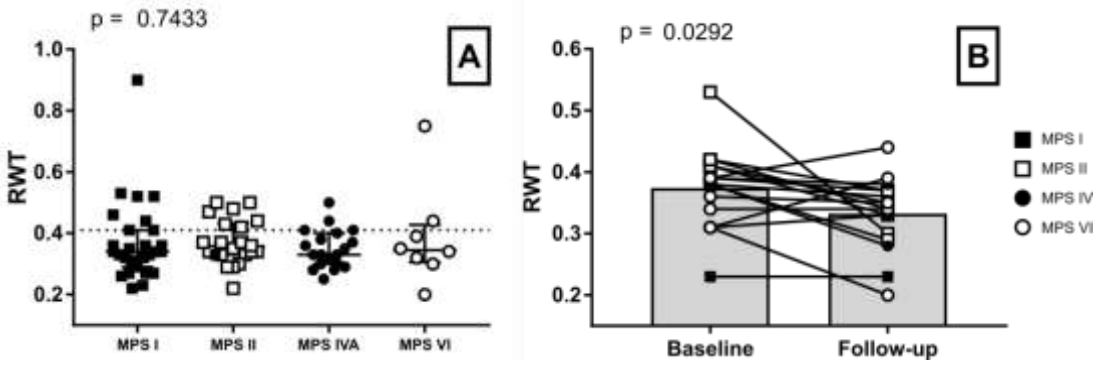
Supplementary table 1. Prevalence of echocardiographic abnormalities at the last available echocardiogram, including both ERT treated and untreated subjects.

	Overall (n=76)	MPS I (n=27)	MPS II (n=22)	MPS IV (n=19)	MPS VI (n=8)	*p*-value
LVM Z score > 2	6/76 (8%)	2/27 (7%)	2/22(9%)	2/19 (11%)	0/8 (0%)	0.821
RWT > 0.41	19/76 (25%)	7/27 (26%)	7/22 (32%)	3/19 (16%)	2/8 (25%)	0.701
Concentric remodeling	16/76 (21%)	5/27 (19%)	6/22 (27%)	3/19 (16%)	2/8 (25%)	0.697
Concentric hypertrophy	3/76 (4%)	0/27 (0%)	1/22 (5%)	2/19 (11%)	0/8 (0%)	
Eccentric hypertrophy	3/76 (4%)	2/27 (7%)	1/22 (5%)	0/19 (0%)	0/8 (0%)	
LAD Z score > 2	20/76 (26%)	7/27 (26%)	4/22 (18%)	7/19 (37%)	2/8 (25%)	0.605
SPAP > 35 mmHg *	11/40 (28%)	6/14 (43%)	2/12 (17%)	1/9 (11%)	2/5 (40%)	0.265
LVEF <55%	1/76 (1%)	0/27 (0%)	0/22 (0%)	1/19 (5%)	0/8 (0%)	0.385

LAD: Left atrial diameter. LVEF: Left ventricle ejection fraction. LVM: left ventricle mass. SPAP: systolic pulmonary artery pressure. * SPAP was measured in 40 of the total 76 available last echocardiograms of the participants. Statistical analysis with chi-squared.



Supplementary Figure 1. Comparison of valve abnormalities before and after ERT. In the statistical analysis, with Wilcoxon matched-pairs signed rank test, no significant difference was identified.



Supplementary Figure 2. Comparison of measurements of relative wall thickness among MPS types (A) as well as before and after ERT for single individuals (B). Statistical analysis with Kruskal-Wallis and Dunn's post-hoc test for comparisons among MPS types (A) and with paired t test for before and after analyses (B).

Capítulo 6:

Artigo 3 – Safety and tolerability of losartan for the treatment of cardiovascular disease in patients with mucopolysaccharidosis type IVA: a preliminary report from a phase 2 randomized clinical trial

Manuscrito em preparação

Safety and tolerability of losartan for the treatment of cardiovascular disease in patients with mucopolysaccharidosis type IVA: Report from a phase 2 randomized clinical trial

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Summary

The mucopolysaccharidoses (MPS) are multisystem disorders caused by deficiencies of lysosomal enzymes responsible for the catabolism of glycosaminoglycans (GAGs). The currently available treatments for MPS were shown to have a limited impact in some manifestations of the disease, including cardiovascular complications. Losartan has been shown to improve cardiac manifestations in mice with MPS. Here, we report the safety and tolerability of losartan in the first four patients with MPS IVA that completed their participation in a randomized, parallel, double-blind, phase 2 clinical trial. The patients were treated with either placebo (one patient) or losartan (three patients) for a period of 12 months and the occurrence of adverse events was recorded. Echocardiograms were obtained at baseline and at the final visit. A total of 16 adverse events (AEs) were reported, 13 in the losartan group (4,3 per patient) and 3 in the placebo group (table 2). None of the AEs were grade 3 or higher. Among the echocardiographic parameters analyzed, we identified potential improvements in E/A and E/e' ratios in patients on treatment with losartan. Our results point to a good safety and tolerability profile of losartan in patients with MPS IVA and supports the evaluation of a larger number of patients in order to clarify its potential benefits.

Registration trial database: The trial was registered in the Clinical Trials Database (registration number NCT03632213)

Keywords: Aortic Root Dilatation; Mucopolysaccharidosis IVA; Left ventricular hypertrophy; Losartan.

Introduction

The mucopolysaccharidoses (MPS) are multisystem disorders caused by deficiencies of lysosomal enzymes responsible for the catabolism of glycosaminoglycans (GAGs). Enzyme replacement therapy (ERT) and, sometimes, hematopoietic stem cell transplantation (HSCT) may be employed in order to restore enzyme activity and avoid the damages caused by the accumulation of GAGs. In spite of that, both ERT or HSCT were shown to have a limited impact in some manifestations of the disease, including cardiovascular complications.

Recently, dysregulation of TGF-β pathway has been implicated in the pathophysiology of aortic and valvular disease of MPS (Ma et al. 2008; Yano et al. 2009; Lyons et al. 2011; Yano et al. 2013). Furthermore, losartan, which modulates its activation, has been shown to attenuate cardiovascular manifestations, including aortic root dilatation, in mice with MPS I (Osborn et al. 2016; Gonzalez et al. 2017). On the basis of those encouraging results, an ongoing clinical trial is now evaluating the safety and efficacy of losartan in MPS disorders, which is focusing on MPS IVA and MPS VI, two types of MPS were aortic root dilatation are especially prevalent in humans. Here, we aimed to report the safety and tolerability of losartan in the first four patients with MPS IVA that completed their participation in the study.

Materials and Methods

This is a preliminary analysis from a randomized, parallel, double-blind, phase 2 clinical trial with the objective of assessing the safety and efficacy of losartan in patients with MPS IVA and MPS VI (ClinicalTrials.gov Identifier: NCT03632213). Eligible research participants have been randomly assigned to receive either losartan 25 mg or placebo.

The study duration is 12 months and its primary outcome measurement is the frequency of adverse events among the groups. Secondary outcome measurements are the reduction over time in the Z score of maximal ARD measured by Valsalva sinus as well as changes of other echocardiographic parameters including ventricular-vascular coupling (Antonini-Canterin et al. 2013) , ejection fraction, global longitudinal strain Z scores (Dallaire et al. 2016), E/A and E/e' ratios and mitral and aortic flows.

Results

A total of ten patients were assessed for eligibility. Three patients declined to participate and six were randomized, being one with MPS VI and five with MPS IVA. In this early report, we assess the observations in the first four patients with MPS IVA that completed the study procedures (figure 1).

All four patients included were females of Latin-American descent and had severe, rapidly progressive phenotype, including prominent skeletal deformities, early onset growth arrest, mild corneal opacity and moderate to severe hearing loss. Three patients were on ERT and one patient was ERT-naïve. No patient reported any symptoms related to cardiovascular disease in the baseline assessment. Table 1 summarizes the clinical characteristics of the patients included in this analysis.

Safety and tolerability

A total of 16 adverse events (AEs) were reported, 13 in the losartan group (4,3 per patient) and 3 in the placebo group (table 2). None of the AEs were grade 3 or higher.

During the study period, the patient on placebo reported an insidious worsening of the gait, that did not resolve after the end of the study. She already had a medical history of gait impairment due to cervical spinal cord compression and musculoskeletal deformities, including genu valgum. She also presented a mild hyperkalemia, which normalized in the follow-up test and did not require treatment, as well as a transitory eosinophilia. All AEs in the losartan group resolved during the study duration and their hematological or biochemical tests did not have clinically significant abnormalities.

Blood pressure remained in normal range during all study visits (figure 2). Nevertheless, subject 05 reported the occurrence of hypertension during measurement in the infusion center, where she receives the ERT. Those high blood pressure measurements were observed for about 2 months.

Treatment was maintained after all adverse events, with the exception of the lumbar pain, which motivated the patient 03 to interrupt temporarily the study drug. As the event did not abate after drug interruption and new laboratory tests were normal, she restarted the study drug and the lumbar pain resolved after a few weeks.

Secondary outcomes

All patients had mitral and/or aortic valve thickening without significant valve insufficiency or stenosis at the baseline and no significant change was observed in the follow-up period. Aortic root dilatation Z score remained relatively stable during the study duration (figure 3A). Measurements related to systolic function, including global longitudinal strain (GLS) and left ventricle ejection fraction (LVEF) fluctuated within normal levels, with a reduction in values of GLS Z score and LVEF observed for subject 2 (figure 3B and 3D). Ventricular

vascular index improved in subject 3 and had a mild reduction in all other participants (figure 3C).

As for the diastolic function, we observed an improvement in E/e' ratio for the patients on losartan (figures 3E-F), as well as a reduction in E/A ratio for subjects 2 and 3. A comparison of the diastolic parameters between subject 01 (on placebo) and subject 02 (on losartan and ERT-naïve) is provided in figure 4.

Discussion

This study analyses, for the first time, the safety and tolerability of losartan as a treatment for cardiovascular manifestations in patients with MPS. In the general population, losartan is a safe and well-tolerated drug, , with the main reported AEs being hypotension, hyperkalemia, reversible renal impairment and teratogenesis (Ritter et al., 2020). However, although ARBs have been used in clinical practice for treatment of heart failure in patients with MPS (Golda et al. 2013), it is still not proposed for treatment of those with asymptomatic, early-stage disease, a setting where a risk-benefit analysis is even more necessary. In this study alladverse events were mild or moderate. These preliminary results suggest that losartan has a favorable safety profile among patients with MPS IVA.

We previously showed that treatment with losartan is able to decrease aortic root diameter and improve cardiac function in MPS I mice (Gonzalez et al. 2017). As aortic root dilatation is usually more severe in MPS IVA, when compared to other MPS types (Poswar et al. 2019), the evaluation of losartan impact seems to be even more promising in this type of MPS. However, it is known that aortic root dilatation has a slow progression rate in MPS IVA(Kampmann et al. 2016b; Lin et al. 2018; Poswar et al. 2019) and the study duration of

12 months was expected to be relatively short for the assessment of significant changes, since the mean aortic root diameter z score slope is estimated to be less than +0.1 in this period (Poswar et al. 2019). Thus, we also relied in other echocardiographic changes to estimate the impact of losartan in the secondary outcomes.

Among the cardiovascular parameters analyzed, we identified potential improvements in E/A and E/e' ratios in patients on treatment with losartan. The E/A ratio measures the transmitral flow profile and the relative contributions of the early (E) and the atrial (A) waves, while E to early diastolic mitral annular tissue velocity (E/e') estimates the left ventricle filling pressures (Mitter et al. 2017). Although a reduction or an increase in the E/A ratio need to interpreted in the appropriate context of other measurements, a reduction of E/e' suggests an improvement in diastolic function. Subtle abnormalities of diastolic function have been described in patients with MPS (Braunlin and Wang 2016; Nijmeijer et al. 2019) and its occurrence may be of clinical relevance since preclinical diastolic dysfunction may progress to heart failure with preserved ejection fraction (HFpEF), a clinical syndrome that accounts for more than half of the cases of heart failure (Correa de Sa et al. 2010; Mitter et al. 2017).

In summary, our results point to a good safety and tolerability profile of losartan in patients with MPS IVA. It also explores potential benefits in echocardiographic parameters, with improvements being identified mostly in the diastolic function, an effect that may be clarified with the evaluation of a larger number of patients.

Competing interests

The authors declare that they have no conflict of interest.

Contributions of individual authors

RG, FP, AS, LG and GB designed research. FP, RG and GB drafted the manuscript. All authors reviewed and approved the final manuscript.

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Ethical approval

The study was approved by the local institutional review board (17-0685; Grupo de Pesquisa e Pós Graduação, Hospital de Clínicas de Porto Alegre, Porto Alegre, Brazil). All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. This article does not contain any studies with animals performed by any of the authors.

Availability of data and materials

The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

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Tables

Table 1. Characteristics of the subjects at the enrollment

	Placebo Group		**Losartan Group**	
Study ID	01	02	03	05
Sex	Female	Female	Female	Female
Age (years)	11	28	35	17
Weight (kg)	19.4	23.45	21.65	33.4
Height (cm)	88.4	103.5	97.8	113.6
BSA (m^2)	0.71	0.83	0.78	1.05
Treated with ERT	Yes	No	Yes	Yes
Age at ERT start (years)	8	N/A	34	13
Sleep apnea	No	No	No	Yes
Hypertension	No	No	No	No
Heart failure	No	No	No	No

ERT, enzyme replacement therapy. N/A, not applicable.

Table 2. Adverse events

	Placebo	**Losartan**
n	01	03
Total number of AEs	3	13
Number of different AEs	3	11
Hyperkalemia	1*	0
Gait disturbance	1†	0
Eosinophilia	1*	0
Upper airway infection	0	3**
Headache	0	1†
Tremor	0	1†
Somnolence	0	1*
Pneumonia	0	1†
Hypertension	0	1†
Lumbar pain	0	1†
Urinary tract infection	0	1†
Muscle cramp	0	1†
Allergic reaction	0	1†
Diarrhea	0	1*

AE, adverse event. * Events were grade 1 - mild. ** Events were grade 1 or 2 (mild or moderate). † Events were grade 2 – moderate.

Figures

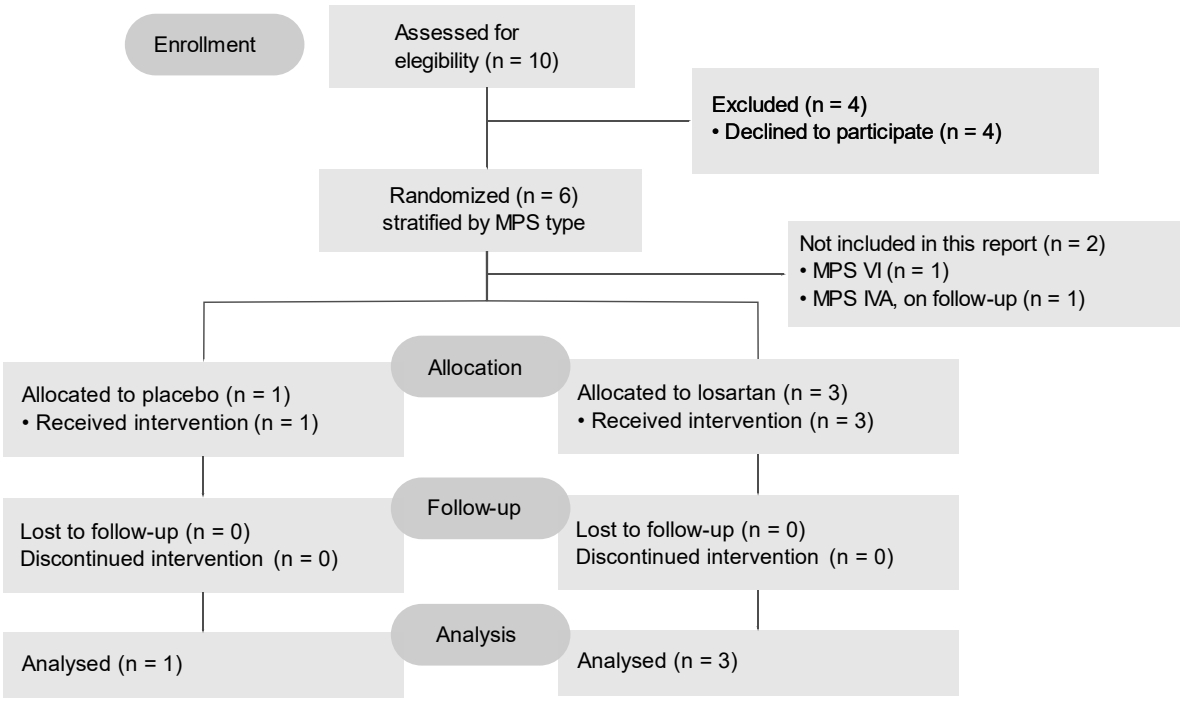

Figure 1. Study flow chart. One patient with MPS VI completed the study procedures but is not included in this early report. Another patient with MPS IVA is currently on follow-up.

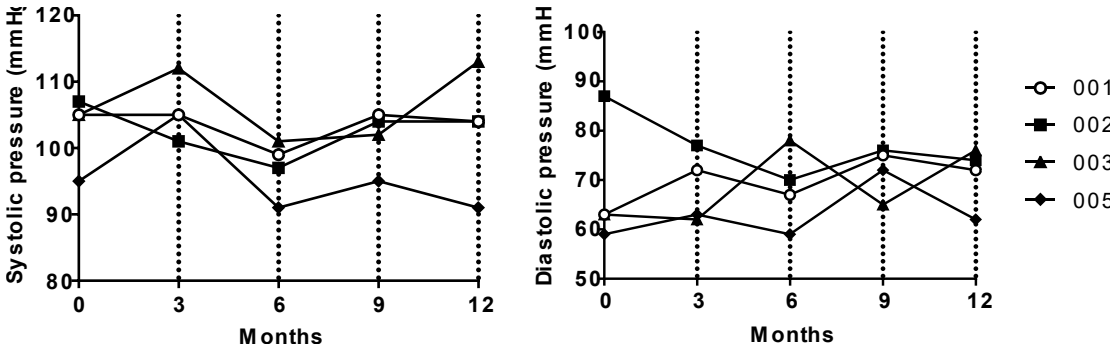

Figure 2. Systemic arterial pressure measurements at study visits.

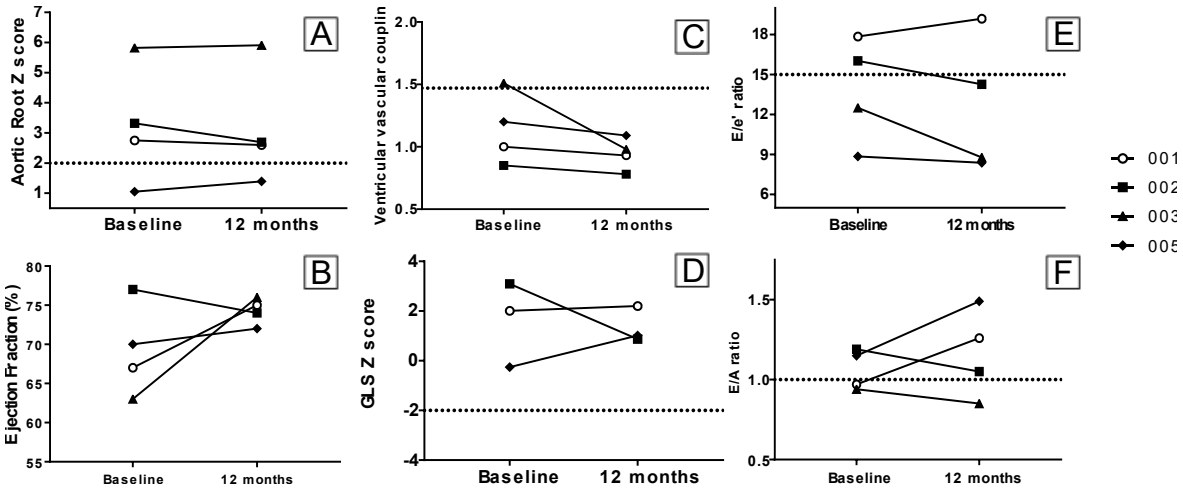

Figure 3. Cardiovascular parameters at baseline and after 12 months of treatment. For subject 03, measurement of global longitudinal strain was not possible due to poor acoustic window. GLS, Global longitudinal strain.

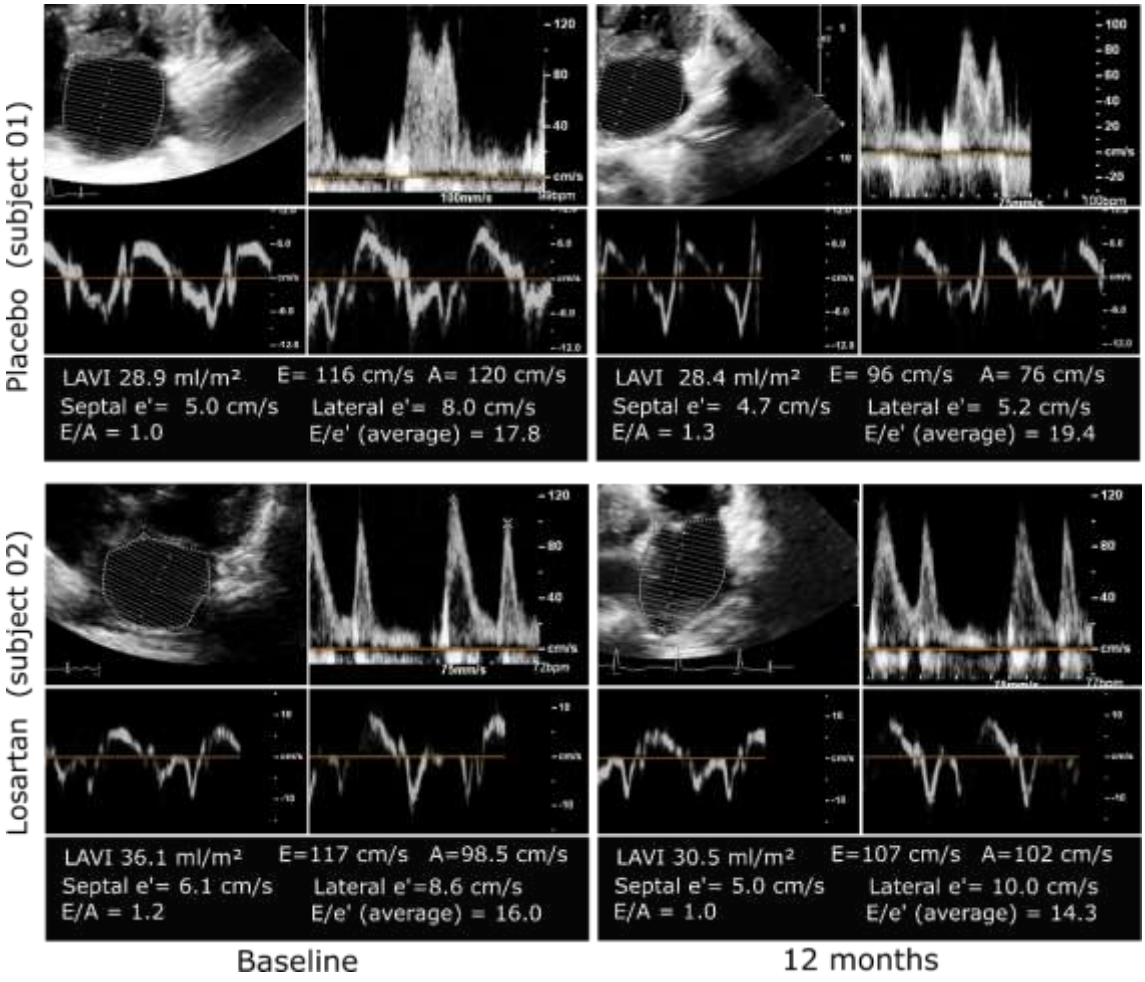

Figure 4. Comparison of diastolic function parameters at baseline and after 12 months between subjects 01 and 02. A decrease of E/A and E/e' ratios in subject 02 is accompanied by a reduction in left atrium volume index (LAVI).

Capítulo 7:
Considerações finais

As manifestações cardiovasculares das MPS vêm sendo progressivamente melhor caracterizadas, assim como o impacto e as limitações das terapias atualmente disponíveis para o tratamento ou prevenção do agravamento.

No primeiro artigo desta tese (capitulo 3), discutimos a alta prevalência da DRA, um achado que embora frequente, nem sempre é reconhecido clinicamente, uma vez que a faixa de normalidade para essa medida é altamente variável de acordo com a idade e outras características do paciente (Campens et al. 2014). Utilizando apenas casos atendidos em um único centro de referência, esse trabalho envolveu o maior número de pacientes até então avaliado especificamente para essa condição e confirmou uma alta prevalência da DRA em pacientes com MPS IVA, MPS VI e formas graves de MPS I. Nesse trabalho, não incluímos nenhum paciente com MPS III, uma vez que a maioria dos pacientes acompanhados no serviço não tinha realizado ecocardiograma. Tal fato se deve provavelmente por este tipo de MPS estar associado a um quadro somático mais brando em relação às outras MPS. Mais recentemente, outros autores não encontraram uma diferença significativa do diâmetro da raiz da aorta entre os pacientes com MPS III e os controles (Nijmeijer et al. 2019).

Uma questão relevante a ser discutida a partir desses resultados é o papel do tipo específico de GAG acumulado nos diferentes tipos de MPS e a ocorrência e gravidade da dilatação de raiz da aorta. O acúmulo de dermatan sulfato, verificado nas MPS I, II e VI, tem sido relacionado à maior gravidade das alterações valvares nesses tipos de MPS, quando comparados à MPS III e a MPS IVA (Leal et al. 2010). Por outro lado, no caso da DRA, é possível que o acúmulo de condroitin-6-sulfato (C6S), verificado na MPS IVA, também tenha um papel etiológico relevante, uma vez que a aorta é um dos principais locais em que o C6S é expresso (Shimada et al. 2014).

No segundo artigo (capítulo 4), expandimos ainda mais a amostra de pacientes avaliados no primeiro estudo e analisamos diversos parâmetros ecocardiográficos e eletrocardiográficos dos pacientes com MPS. Os dados gerados por este estudo trazem dados adicionais importantes relacionados ao fenótipo cardíaco das MPS, podendo auxiliar profissionais de saúde e pesquisadores a compreender melhor o espectro de alterações cardiovasculares presentes nos pacientes com MPS I, II, IVA e VI Além disso, uma análise comparativa entre os dados gerados neste estudo e os produzidos em avaliações de modelos animais poderá auxiliar na tradução dos resultados de estudos pré-clínicos durante o desenho

de novos estudos com seres humanos.Por exemplo, enquanto uma redução de fração de ejeção tem sido reportada em modelos murinos de MPS (Jordan et al. 2005) e o uso de certos medicamentos tem resultado em melhora desse parâmetro (Gonzalez et al. 2017; Gonzalez et al. 2018), essa alteração foi infrequente em nosso trabalho, o que sugere que a mudança na fração de ejeção seja um desfecho pouco informativo para avaliação em ensaios clínicos em pacientes com MPS.

Tanto no primeiro, como no segundo artigo, utilizamos os escores Z ajustados pela superfície corporal para determinar a normalidade relativa às medidas ecocardiográficas. A superfície corporal tem como limitação a ausência de reajuste por adiposidade, uma vez que o tecido adiposo tem menor atividade metabólica (Frayn et al. 2003). Entretanto, ainda assim, consideramos que o escore Z ajustado pela superfície corporal seja a melhor medida a ser utilizada mesmo em pacientes com MPS, uma vez que há uma relação linear entre débito cardíaco e superfície corporal. O débito cardíaco parece ser em si uma força determinante do tamanho das estruturas cardiovasculares, que são otimizadas de modo a reduzir a demanda energética necessária para impulsionar o sangue (Gutgesell and Rembold 1990).

Além da superfície corporal, a variabilidade da frequência cardíaca também tem impacto na maior demanda de débito cardíaco. Assim, crianças maiores, que realizam mais atividades físicas, devem ter uma maior variação da frequência cardíaca, resultando em maior demanda energética e maiores valores mensurados quando comparadas a lactentes (Sluysmans and Colan 2016). Ao contrário, é esperado que crianças restritas a cadeiras de rodas (como algumas das crianças com MPS) apresentem menor variabilidade da frequência cardíaca, resultando em estruturas cardíacas relativamente menores. Tais variáveis não foram ainda incorporadas às fórmulas de escore Z atualmente disponíveis.

Com a disponibilidade da TRE como principal estratégia terapêutica para as MPS, uma melhor compreensão de seus efeitos é essencial para o acompanhamento dos pacientes e mesmo para o desenvolvimento de novas terapias. No caso das manifestações cardiovasculares, no primeiro e no segundo artigo desta tese, nossos achados confirmam que a TRE, embora promova uma melhora dos parâmetros relacionados a hipertrofia ventricular esquerda, tem um papel limitado para a maior parte das manifestações cardiovasculares. Tais

achados reforçam a necessidade de desenvolvimento de novas terapias que superem essas limitações.

Com financiamento do Hospital de Clínicas de Porto Alegre (FIPE-GPPG) e de uma fundação canadense destinada ao fomento de pesquisas para a MPS VI (Isaac Foundation), foi planejado um ensaio clínico para avaliar a segurança e a eficácia da losartana nas MPS como terapia auxiliar à TRE, cujos resultados preliminares foram discutidos no artigo 3 (capítulo 5). A escolha desse fármaco se baseou principalmente nos resultados promissores de outra pesquisa vinculada ao PPGBM/UFRGS (Gonzalez et al. 2017).

A execução de uma pesquisa clínica envolvendo uma doença rara, porém, traz grandes desafios relacionados ao recrutamento dos pacientes. Dentro das possibilidades de orçamento, tivemos de optar por um estudo em único centro e nos deparamos com a baixa prevalência de MPS VI em nosso estado, com apenas quatro pacientes com esse diagnóstico em todo o território estadual. Além disso, com a irregularidade de recebimento da TRE em nosso meio, a exigência inicial de que os pacientes estivessem há pelo menos seis meses com TRE regular não se mostrou viável e, mesmo a reduzindo para três meses esse período, tivemos de adiar a inclusão de alguns pacientes pelo desabastecimento generalizado dos medicamentos nas secretarias de saúde.

Ainda assim, mesmo considerando as limitações relativas ao baixo número de pacientes incluídos nesta análise, os resultados parciais discutidos no terceiro artigo desta tese trazem dados sobre o uso da losartana nas MPS, importantes para a continuidade da sua avaliação como estratégia terapêutica. A modulação de vias de sinalização celulares, através do uso da losartana ou outras moléculas pequenas, permanece como uma área promissora para o desenvolvimento de novos tratamentos para as MPS e outras DLs.

Capítulo 8:
Conclusões

A DRA foi detectada em pacientes com diferentes tipos de MPS, sendo mais prevalente em paciente com MPS IVA e VI. Especialmente em pacientes com MPS IVA confirmou-se a característica progressiva da dilatação. A TRE parece ter pouco efeito sobre este parâmetro.

As manifestações cardiovasculares nos pacientes com MPS são bastante variáveis, estendendo-se de um acometimento leve com espessamento das válvulas sem repercussão funcional até um quadro avançado de hipertrofia ventricular esquerda e insuficiência cardíaca com fração de ejeção reduzida. A TRE leva a uma redução significativa da hipertrofia ventricular, mas um efeito marcante sobre outras manifestações cardiovasculares não foi identificado.

A losartana demonstrou ter um perfil de segurança favorável em pacientes com mucopolissacaridose IVA. Os resultados até o momento não permitem uma definição concreta sobre seu impacto terapêutico em pacientes com MPS, embora possam sugerir uma melhora em parâmetros relacionados à função diastólica.

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Apêndices

1. Apêndice 1 – Aprovação do Comitê de Ética em Pesquisa – Projeto 1

HCPA - HOSPITAL DE CLÍNICAS DE PORTO ALEGRE
GRUPO DE PESQUISA E PÓS-GRADUAÇÃO

COMISSÃO CIENTÍFICA

A Comissão Científica do Hospital de Clínicas de Porto Alegre analisou o projeto:

Projeto: 170013
Data da Versão do Projeto: 06/01/2017

Pesquisadores:
GUILHERME BALDO
FABIANO DE OLIVEIRA POSWAR
ROBERTO GIUGLIANI

Título: Avaliação da função cardíaca em pacientes com MPS

Este projeto foi APROVADO em seus aspectos éticos, metodológicos, logísticos e financeiros para ser realizado no Hospital de Clínicas de Porto Alegre.
Esta aprovação está baseada nos pareceres dos respectivos Comitês de Ética e do Serviço de Gestão em Pesquisa.

- Os pesquisadores vinculados ao projeto não participaram de qualquer etapa do processo de avalição de seus projetos.

- O pesquisador deverá apresentar relatórios semestrais de acompanhamento e relatório final ao Grupo de Pesquisa e Pós-Graduação (GPPG)

Porto Alegre, 25 de maio de 2017.

Prof. José Roberto Goldim
Coordenador CEP/HCPA

2. Apêndice 2 – Aprovação no comitê de ética – projeto 2

HCPA - HOSPITAL DE CLÍNICAS DE PORTO ALEGRE
GRUPO DE PESQUISA E PÓS-GRADUAÇÃO

CARTA DE APROVAÇÃO

Projeto: 170685
Data da Versão do Projeto: 09/02/2018

Pesquisadores:
ROBERTO GIUGLIANI
GUILHERME BALDO
LUCIANA GIUGLIANI
FERNANDO MACHADO DA COSTA
LUZ ELENA DURÁN CARABALI
MARCELA MIGLIAVADA PEREIRA DIAS
FABIANO DE OLIVEIRA POSWAR
MARINA BAUER ZAMBRANO
SIRLEI KASPRCZAK MARTINS

Título: UM ENSAIO CLÍNICO RANDOMIZADO PARA AVALIAR OS EFEITOS DA LOSARTANA NA DOENÇA CARDIOVASCULAR EM PACIENTES COM MUCOPOLISSACARIDOSES IVA E VI

Este projeto foi APROVADO em seus aspectos éticos, metodológicos, logísticos e financeiros para ser realizado no Hospital de Clínicas de Porto Alegre.
Esta aprovação está baseada nos pareceres dos respectivos Comitês de Ética e do Serviço de Gestão em Pesquisa.

- Os pesquisadores vinculados ao projeto não participaram de qualquer etapa do processo de avalição de seus projetos.

- O pesquisador deverá apresentar relatórios semestrais de acompanhamento e relatório final ao Grupo de Pesquisa e Pós-Graduação (GPPG)

Porto Alegre, 09 de abril de 2018.

Profª. Patricia Ashton Prolla
Coordenadora GPPG/HCPA

Anexos

1. Anexo 1 - Artigo publicado durante o período do doutorado, relacionado com a tese: Lysosomal diseases: Overview on current diagnosis and treatment

Publicado na revista Genetics and Molecular Biology.

Genetics and Molecular Biology Online Ahead of Print

DOI: http://dx.doi.org/10.1590/1678-4685-GMB-2018-0159

Lysosomal diseases: Overview on current diagnosis and treatment

Fabiano de Oliveira Poswar[1,3*], Filippo Vairo[4,5*], Maira Burin[1], Kristiane Michelin-Tirelli[1], Ana Carolina Brusius-Facchin[1], Francyne Kubaski[1], Carolina Fischinger Moura de Souza[1], Guilherme Baldo[1,6,7] and Roberto Giugliani[1,2,3]

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Abstract

Lysosomal diseases (LDs), also known as lysosomal storage diseases (LSDs), are a heterogeneous group of conditions caused by defects in lysosomal function. LDs may result from deficiency of lysosomal hydrolases, membrane-associated transporters or other non-enzymatic proteins. Interest in the LD field is growing each year, as more conditions are, or will soon be treatable. In this article, we review the diagnosis of LDs, from clinical suspicion and screening tests to the identification of enzyme or protein deficiencies and molecular genetic diagnosis. We also cover the treatment approaches that are currently available or in development, including hematopoietic stem cell transplantation, enzyme replacement therapy, small molecules, and gene therapy.

Keywords: Lysosomal storage diseases, neonatal screening, hematopoietic stem cell transplantation, enzyme replacement therapy, gene therapy.

Received: June 15, 2018; Accepted: October 30, 2018.

Introduction

Lysosomes are membrane-bound organelles, which contain, among other components, hydrolytic enzymes that operate in an acidic environment (Sabatini and Adesnik, 2014). Lysosomes are capable of digesting all types of macromolecules and participate in the breakdown of both extracellular and intracellular components that are targeted to them through the processes of endocytosis or autophagy, respectively.

Lysosomal diseases, also known as lysosomal storage diseases, are a heterogeneous group of diseases caused by defects in lysosomal function (Valle *et al.*, 2014). Most LDs result from a deficiency in lysosomal hydrolases (*e.g.*, alpha-galactosidase in Fabry disease). Alternatively, LDs may be caused by deficiencies in lysosomal membrane-associated transporters (*e.g.*, cystinosin in cystinosis) or other non-enzymatic proteins (*e.g.*, CLN3 in Batten disease). According to the WORLDSymposia® official list of lysosomal diseases, 66 clinical conditions related to 53 distinct genes are recognized as LDs (WORLDSymposium, 2018).

Although individually very rare, the incidence of LDs as a group is estimated to be as high as 1 in 4000 in some countries (Giugliani *et al.*, 2017a). The exact prevalence is difficult to estimate, considering the clinical heterogeneity of LDs, which may lead to missed diagnoses. According to Medical Genetics Service of the Hospital de Clínicas de Porto Alegre data, the investigation of high-risk subjects led to 3,512 LD diagnoses in Brazil from 1982 to 2017 (Table 1).

Interest in the LD field is growing as more conditions are now treatable or are expected to be treatable in the near future by distinct approaches including hematopoietic stem cell transplantation, enzyme replacement, small molecules, and gene therapy (Beck, 2018). Research in this field is also

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2. Anexo 2 - Artigo publicado durante o período do doutorado, relacionado com a tese: Neurological manifestations of lysosomal disorders and emerging therapies targeting the CNS

Publicado na revista Lancet Child Adolesc Health 2018; 2: 56–68

Review

Neurological manifestations of lysosomal disorders and emerging therapies targeting the CNS

Roberto Giugliani, Filippo Vairo, Francyne Kubaski, Fabiano Poswar, Mariluce Riegel, Guilherme Baldo, Jonas Alex Saute

Lancet Child Adolesc Health 2018; 2: 56–68

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Medical Genetics Service, Hospital de Clínicas de Porto Alegre, Porto Alegre, Brazil (Prof R Giugliani MD, F Poswar MD, M Riegel PhD, J A Saute MD); Department of Genetics (Prof R Giugliani), Postgraduate Program in Genetics and Molecular Biology (Prof R Giugliani, F Poswar, M Riegel, G Baldo PhD), Postgraduate Program in Physiology (G Baldo), and Postgraduate Program in Medicine: Medical Sciences (J A Saute), Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil; Mayo Clinic, Rochester, MN, USA (F Vairo MD); and University of Delaware, Newark, DE, USA (F Kubaski PhD)

Lysosomal disorders have been an area of interest since intravenous enzyme replacement therapy was successfully introduced for the treatment of Gaucher's disease in the early 1990s. This treatment approach has also been developed for several other lysosomal disorders, including Fabry's disease, Pompe's disease, lysosomal acid lipase deficiency, and five types of mucopolysaccharidosis. Despite the benefits of enzyme replacement therapy, it has limitations—most importantly, its ineffectiveness in treating the neurological components of lysosomal disorders, as only a small proportion of recombinant enzymes can cross the blood–brain barrier. Development of strategies to improve drug delivery to the CNS is now the primary focus in lysosomal disorder research. This Review discusses the neurological manifestations and emerging therapies for the CNS component of these diseases. The therapies in development (which are now in phase 1 or phase 2 clinical trials) might be for specific lysosomal disorders (enzyme replacement therapy via intrathecal or intracerebroventricular routes or with fusion proteins, or gene therapy) or applicable to more than one lysosomal disorder (haemopoietic stem cell transplantation, pharmacological chaperones, substrate reduction therapy, or stop codon readthrough). The combination of early diagnosis with effective therapies should change the outlook for patients with lysosomal disorders with neurological involvement in the next 5–10 years.

Introduction

Lysosomal disorders are mainly caused by mutations in genes encoding lysosomal hydrolases, which are responsible for the degradation of macromolecules inside cells.[1] Defects in the post-translational processing of lysosomal enzymes, enzyme trafficking and targeting, enzyme cofactors, and the function of non-enzymatic lysosomal transmembrane and soluble proteins also cause cellular disturbances, leading to lysosomal disorders.[2]

Classification of lysosomal disorders is generally based on either the biochemical nature of the storage material or the underlying disease-causing mechanism.[1] The former classification, although clinically useful, does not take into account that several different substrates are stored in many lysosomal disorders, whereas the latter is more suitable for the study of disease pathogenesis and design of new therapies. Table 1 shows an adapted classification based on both models.[3–21]

Although lysosomal enzymes are products of housekeeping genes that are expressed ubiquitously, their substrates have a much less uniform distribution across cell types. Substrate expression, therefore, has a key role in the selectivity of tissues affected by storage.[1] In sphingolipidoses, for example, the cell can only store a specific glycosphingolipid if it synthesises it or acquires it from an external source. For instance, in type 1 Gaucher's disease, the lack of glucocerebrosidase activity leads to storage of glucosylceramide—which is acquired following phagocytosis of senescent leucocytes—in macrophages. In lysosomal disorders that affect the CNS, a similar process, such as apoptotic cell clearance, could lead to storage in microglial cells, whereas storage in neurons arises because they synthesise the lipid in question. The pattern of storage in the CNS is cell-type dependent, as different neurons have different glycosphingolipid expression profiles, and pathology is therefore confined to certain cell populations.

Differences in cellular vulnerability to substrate accumulation and to other cellular dysfunctions related to lysosomal disorders also determine which tissues are affected.[22] CNS involvement occurs in two-thirds of lysosomal disorders, despite a lower concentration of storage materials in the brain than in other organs.[2,22] Neuronal vulnerability is probably related to the lack of compensatory cellular metabolic pathways and to limited cell regeneration.[2] Storage in the CNS might also affect glial cells, resulting in abnormal formation or maintenance of myelin, astrocytosis, and activation of microglia, which can be key mediators of neurodegeneration.

Key messages

- Lysosomal disorders are multisystemic and progressive conditions, and many of them have neurological involvement, which often dominates disease presentation.
- Intravenous enzyme replacement therapy, which is available for several lysosomal disorders, does not address the neurological component of the disease, as the recombinant enzymes do not cross the blood–brain barrier effectively.
- Several novel therapeutic strategies (including intrathecal or intracerebroventricular delivery of enzymes, fusion proteins that cross the blood–brain barrier, substrate reduction therapy, pharmacological chaperones, and gene therapy) are being developed to treat the neurological component of many lysosomal disorders, and should become available soon.
- Knowledge about the natural history of the neurological manifestations of lysosomal disorders is important to define outcomes for clinical trials, and the identification of the minimally important differences will be paramount for assessment of long-term and short-term efficacies of new therapies for the different lysosomal disorders.
- The combination of early diagnosis with effective therapies should change the outlook for patients with lysosomal disorders with neurological involvement in the next 5–10 years.

Neurological manifestations

Lysosomal disorders are often multisystem disorders, although in some neuronopathic subtypes disease

3. Anexo 3 – Artigo publicado durante o período do doutorado, relacionado com a tese: Diagnosis of mucopolysaccharidoses

Publicado na revista Diagnostics 2020; 10: 172

diagnostics

MDPI

Review

Diagnosis of Mucopolysaccharidoses

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Received: 31 January 2020; Accepted: 18 March 2020; Published: 22 March 2020

Abstract: The mucopolysaccharidoses (MPSs) include 11 different conditions caused by specic enzyme deciencies in the degradation pathway of glycosaminoglycans (GAGs). Although most MPS types present increased levels of GAGs in tissues, including blood and urine, diagnosis is challenging as specic enzyme assays are needed for the correct diagnosis. Enzyme assays are usually performed in blood, with some samples (as leukocytes) providing a nal diagnosis, while others (such as dried blood spots) still being considered as screening methods. The identication of variants in the specic genes that encode each MPS-related enzyme is helpful for diagnosis conrmation (when needed), carrier detection, genetic counseling, prenatal diagnosis (preferably in combination with enzyme assays) and phenotype prediction. Although the usual diagnostic ow in high-risk patients starts with the measurement of urinary GAGs, it continues with specic enzyme assays and is completed with mutation identication; there is a growing trend to have genotype-based investigations performed at the beginning of the investigation. In such cases, conrmation of pathogenicity of the variants identied should be conrmed by measurement of enzyme activity and /or identication and /or quantication of GAG species. As there is a growing number of countries performing newborn screening for MPS diseases, the investigation of a low enzyme activity by the measurement of GAG species concentration and identication of gene mutations in the same DBS sample is recommended before the suspicion of MPS is taken to the family. With specic therapies already available for most MPS patients, and with clinical trials in progress for many conditions, the specic diagnosis of MPS as early as possible is becoming increasingly necessary. In this review, we describe traditional and the most up to date diagnostic methods for mucopolysaccharidoses.

Keywords: mucopolysaccharidoses; glycosaminoglycans; enzyme replacement therapy; tandem mass spectrometry; newborn screening.

1. Introduction

The mucopolysaccharidoses (MPSs) comprises 11 lysosomal diseases in which there is a deciency in a specic step of the degradation of glycosaminoglycans (GAGs). This deciency leads to storage of GAGs in tissues and to a range of clinical consequences, which may include CNS impairment, depending on the specic MPS type [1,2]. Each MPS is clinically heterogeneous, with severe and attenuated cases within each MPS type, a fact that may be related to small variations in the residual enzyme activity, conditioned by the genetic variation present in the patient [3].

4. Anexo 4 – Artigo publicado durante o período do doutorado, relacionado com a tese: Mucopolysaccharidosis type I

Publicado na revista Diagnostics 2020; 10: 160

diagnostics

MDPI

Review

Mucopolysaccharidosis Type I

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Abstract: Mucopolysaccharidosis type I (MPS I) is caused by the deciency of α-L-iduronidase, leading to the storage of dermatan and heparan sulfate. There is a broad phenotypical spectrum with the presence or absence of neurological impairment. The classical form is known as Hurler syndrome, the intermediate form as HurlerScheie, and the most attenuated form is known as Scheie syndrome. Phenotype seems to be largely inuenced by genotype. Patients usually develop several somatic symptoms such as abdominal hernias, extensive dermal melanocytosis, thoracolumbar kyphosis odontoid dysplasia, arthropathy, coxa valga and genu valgum, coarse facial features, respiratory and cardiac impairment. The diagnosis is based on the quantication of α-L-iduronidase coupled with glycosaminoglycan analysis and gene sequencing. Guidelines for treatment recommend hematopoietic stem cell transplantation for young Hurler patients (usually at less than 30 months of age). Intravenous enzyme replacement is approved and is the standard of care for attenuatedHurlerScheie and Scheieforms (without cognitive impairment) and for the late-diagnosed severeHurlercases. Intrathecal enzyme replacement therapy is under evaluation, but it seems to be safe and effective. Other therapeutic approaches such as gene therapy, gene editing, stop codon read through, and therapy with small molecules are under development. Newborn screening is now allowing the early identication of MPS I patients, who can then be treated within their rst days of life, potentially leading to a dramatic change in the diseases progression. Supportive care is very important to improve quality of life and might include several surgeries throughout the life course.

Keywords: mucopolysaccharidosis type I; Hurler syndrome; HurlerScheie syndrome; Scheie syndrome; glycosaminoglycans; enzyme replacement therapy; hematopoietic stem cell transplantation

5. Anexo 5 - Artigo publicado durante o período do doutorado, relacionado com a tese: Phase I and II clinical trials for the mucopolysaccharidoses

Publicado na revista Expert Opinion on Investigational Drugs, 26:12, 1331-1340.

EXPERT OPINION ON INVESTIGATIONAL DRUGS, 2017
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REVIEW

Phase I and II clinical trials for the mucopolysaccharidoses

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ABSTRACT

Introduction: The mucopolysaccharidoses are lysosomal diseases characterized by deficient activity of one of the enzymes that degrades glycosaminoglycans. Treatment options are limited; therefore, new treatments are under investigation.

Areas covered: We review the medicinal products for the treatment of mucopolysaccharidoses that are currently being investigated in phase I and phase II clinical trials.

Expert opinion: The number of alternatives to treat MPS diseases increased dramatically in an attempt to provide therapy options for orphan MPS diseases and to address the unmet needs of the MPS that already have a treatment available. Intravenous enzyme replacement therapy (ERT) with fusion proteins, intrathecal/intracerebroventricular (ICV) ERT and gene therapy are the most promising strategies addressing the CNS manifestations. Stop-codon read-through, although proposed only for patients with nonsense mutations, might be useful in all MPS types. Substrate reduction therapy could also play a role in any MPS type, as anti-inflammatory drugs are also being tested. This new generation of therapies is now in clinical development and should bring new hope to MPS patients. As cost and logistics remain major challenges, especially for low- and middle-income countries, the possibility of having a one-time treatment such as gene therapy is anxiously awaited by affected families and healthcare systems.

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Clinical trials; mucopolysaccharidoses; glycosaminoglycans; enzyme replacement therapy; intrathecal therapy; substrate reduction therapy; stop-codon read through; gene therapy

1. The mucopolysaccharidoses: general aspects

The mucopolysaccharidoses (MPS) are a group of genetic disorders of glycosaminoglycan (GAG; formerly known as mucopolysaccharide) catabolism. Each MPS is caused by mutations in genes that encode lysosomal enzymes, which are involved in the degradation of GAGs. Therefore, these partially degraded substances accumulate within the lysosome, causing cell damage that ultimately leads to organ dysfunction and reduced life expectancy. Patients typically experience the onset of clinical disease during early childhood, and clinical features may include hepatosplenomegaly, hearing and vision impairment, skeletal dysplasia, joint stiffness, airway and cardiac abnormalities, and mental retardation in the severe forms of MPS I, MPS II, all subtypes of MPS III, and MPS VII (Table 1).

Approved treatments available for MPS are basically enzyme replacement therapy (ERT, available for MPS I, II, IVA, and VI) and hematopoietic stem cell transplantation (HSCT), which is a standard of care for MPS I and may be an option for MPS II, MPS IVA, and MPS VI, although it still has limited use in the latter conditions due to the better safety profile of ERT [1].

Both the ERT and the HSCT aim to provide an external source of enzymes, addressing the primary defect of the MPS. The rationale of these therapies was predicted by De Duve in 1964, who described that the lysosome is the destination for any substance that is phagocytized [2].

In the case of ERT, a recombinant human enzyme is produced in a genetically modified cell line or organism and regularly injected in the blood (intravenous route) or the cerebrospinal fluid (intrathecal, ICV, or intracisternal routes). Phagocytosis of the enzyme is performed, in most cases, in a mannose-6-phosphate manner. However, the currently available enzymes are not able to cross the blood–brain barrier and cannot address the central nervous system (CNS) manifestations of the MPS and may also be ineffective for some of the skeletal and cardiovascular manifestations, particularly when the treatment is not started early.

In HSCT, the enzyme is produced by the donor cells and released in the bloodstream to cross-correct other cells. Furthermore, microglial cells derived from the transplanted donor cells provide the CNS with a source of the enzyme and may prevent cognitive deterioration. Nevertheless, HCST still does not prevent progression of skeletal manifestations in MPS I or MPS II, and it was not shown to be effective in MPS III [3]. In addition, despite appearing to be as effective as ERT for MPS IVA [4] and MPS VI [5] treatment, it is not routinely used for these conditions, as the risk-benefit profile of HCST is thought to be unfavorable for MPS disorders without cognitive impairment [6]. For MPS VII, there is also evidence of the benefits of HSCT, but this evidence is anecdotal [7].

Based on these findings, new therapies have arisen. Gene therapy, performed either by a classical approach or by gene

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6. Anexo 6 - Artigo publicado durante o período do doutorado, relacionado com a tese: Intrathecal/Intracerebroventricular enzyme replacement therapy for the mucopolysaccharidoses: efficacy, safety, and prospects

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REVIEW

Intrathecal/Intracerebroventricular enzyme replacement therapy for the mucopolysaccharidoses: efficacy, safety, and prospects

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ABSTRACT

Introduction: The mucopolysaccharidoses (MPS) are lysosomal storage diseases (LSDs) caused by the deficiency of an enzyme involved in the breakdown of glycosaminoglycans (GAGs), which leads to GAG storage and results in multisystemic manifestations. In some of the 11 MPS types, patients could have cognitive involvement and/or secondary neurologic manifestations. Intravenous enzyme replacement therapy (ERT), already approved for several MPS types, is not able to cross the blood–brain barrier and does not address the neurologic manifestations present in most MPS types. Intrathecal (IT) or intracerebroventricular (ICV) administration of the enzyme directly into the cerebrospinal fluid (CSF) has been proposed and experienced in clinical trials and in single cases.

Areas covered: This paper briefly summarizes the development of ERT to treat LSDs, particularly MPS, the technical aspects related to its CSF administration, the experience obtained so far with the IT and ICV use in several MPS types and provides an expert opinion on this subject.

Expert opinion: Treatment of neuropathic MPS remains a challenge. The results of ongoing trials may bring IT and ICV administration of ERT to clinical use in the coming years, making it a therapeutic option for the treatment of neuronopathic patients in several MPS types.

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Mucopolysaccharidoses; enzyme replacement therapy; intrathecal; intracerebroventricular; glycosaminoglycans; blood–brain barrier

1. Introduction

The mucopolysaccharidoses (MPS), a group of lysosomal storage diseases (LSDs), include 11 enzyme deficiencies, each of them involved in one step of the degradation of glycosaminoglycans (GAGs). The deficiency of one of these enzymes leads to the build-up of storage of one or more GAGs, which leads to multisystem manifestations [1].

In some of the MPS (types I, II, IIIA, IIB, IIIC, IIID, and VII), patients may have cognitive involvement. In other types (types IVA and VI), usually do not have cognitive decline, but neurologic manifestations could occur secondary to spinal cord compression [1]. Some patients with MPS IVB can have cognitive involvement [2]. No cognitive manifestations were described in the few cases of MPS IX reported so far [3,4].

Despite bone marrow transplantation was proposed a potential therapy for the MPS, its role has been largely restricted to the severe form of MPS I (Hurler syndrome) and should be performed early in life to provide benefits related to the CNS manifestations [5].

Intravenous enzyme replacement therapy (ERT) was first approved in 2003 for the treatment of MPS I, followed in 2005 and 2006 for the treatment of MPS VI and MPS II, respectively. More recently, it was also approved in 2014 for MPS IVA and in 2017 for MPS VII [6]. One of the main limitations of intravenous (IV) ERT is its inability to cross the blood–brain barrier (BBB) and address the neurologic manifestations present in most MPS types [7]. Modified enzymes (fusion proteins) are being tested to allow the intravenously administered ERT to bypass the BBB and reach the CNS [8,9], but these protocols are still investigational. Considering the limitation of the IV administered enzyme to reach the CNS, the infusion of the enzyme directly in the CSF by intrathecal (IT) or intracerebroventricular (ICV) administration has been proposed and has been experienced in isolated cases and in clinical trials.

This paper will briefly summarize the development of ERT to treat LSDs, and MPS, in particular, the technical aspects related to the administration of ERT directly to the CSF, and the experience obtained so far with the IT and ICV administration of ERT in several MPS types (MPS I, II, IIIA, IIIB, and VI) and will provide an expert opinion on this subject.

2. ERT

2.1. The development of ERT for the MPS

The idea of treating LSDs through supplementation of exogenous enzymes was first conceived by de Duve in 1964 [10]. That possibility was raised after the description of the lysosomes as the final destination of any substance that enters the

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7. Anexo 7 - Artigo publicado durante o período do doutorado, relacionado com a tese: Long-term restoration of alpha-L-iduronidase activity in fibroblasts from patients with mucopolysaccharidosis type I after non-viral gene transfer

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Brief Communication

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Long-term restoration of alpha-L-iduronidase activity in fibroblasts from patients with mucopolysaccharidosis type I after non-viral gene transfer

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ABSTRACT

Mucopolysaccharidosis type I (MPS I) is a lysosomal storage disorder caused by deficiency of alpha-L-iduronidase (IDUA). Limitations such as the need for weekly injections, high morbidity and mortality, and high cost of current treatments show that new approaches to treat this disease are required. In this study, we aimed to correct fibroblasts from a patient with MPS I using non-viral gene therapy. Using a plasmid encoding the human IDUA cDNA, we achieved stable high IDUA levels in transfected fibroblasts up to 6 months of treatment. These results serve as proof of concept that a non-viral approach can correct the enzyme deficiency in cells of patients with lysosomal storage disorders, which can be used as a research tool for a series of disease aspects. Future studies should focus on showing if this approach can be useful in small animals and clinical trials.

Keywords: *Mucopolysaccharidosis I; gene therapy; iduronidase*

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In mucopolysaccharidosis type I (MPS I), the deficiency in alpha-L-iduronidase (IDUA) leads to lysosomal accumulation of the glycosaminoglycans (GAGs) heparan and dermatan sulfates. Abnormal storage of these GAGs results in progressive cellular and multi-organ dysfunction[1].

MPS I has multiple clinical presentations. In its most severe form, also known as Hurler syndrome, the disease, in the first year of age, is characterized by umbilical hernia, hepatosplenomegaly, skeletal abnormalities and developmental delay. The signs are progressive and, when untreated, typically result in death in the first two decades of life. In its mildest form, also known as Scheie syndrome, the disease has its onset in late childhood or puberty with slowly progressive skeletal, heart and eye manifestations. Intermediate forms (i.e., Hurler-Scheie syndrome) also exist[2].

Treatment approaches include hematopoietic stem cell transplantation (HSCT) and enzyme replacement therapy (ERT), both with limited effects. Despite recent advances, HSCT still presents a significant morbidity and mortality risk, and finding compatible donors is difficult. HSCT is also unable to halt the progression of skeletal manifestations even in patients with full engraftment and, currently, is indicated only to patients with the severe form of the disease, under the age of 2-2.5 years[3]. Conversely, ERT does not correct difficult-to-reach organs, such as the brain and the heart valves, and also has limited effects on the skeletal system[4-6]. Hence, novel strategies are needed to treat MPS I.

Gene transfer is a promising option, but safety concerns over the use of viral vectors are still a major problem, since insertional mutagenesis and development of immune response to the virus have already been described[7]. In the present study, we tested a non-viral gene transfer method aimed to correct the enzyme deficiency in fibroblasts from patients with MPS I.

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